ASSESSMENT OF PLATELET INDICES AND ITS PREDICTIVE VALUE IN TYPE 2 DIABETES MELLITUS ADULT PATIENTS ATTENDING AT BISHOFTU GENERAL HOSPITAL, CENTRAL OF ETHIOPIA: A COMPARATIVE CROSS-SECTIONAL STUDY

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A RESEARCH PAPER SUBMITTED TO THE SCHOOL OF MEDICAL LABORATORY SCIENCES, FACULTY OF HEALTH SCIENCES, INSTITUTE OF HEALTH,JIMMA UNIVERSITY, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE MASTERS OF SCIENCE IN CLINICAL LABORATORY SCIENCES SPECIALITY IN HEMATOLOGY AND IMMUNOHEMATOLOGY

JANUARY, 2023

JIMMA, ETHIOPIA

JIMMA UNIVERSITY

INSTITUTE OF HEALTH

FACULTY OF HEALTH SCIENCES

SCHOOL OF MEDICAL LABORATORY SCIENCES

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ABSTRACT

***Background**: Diabetes is a chronic metabolic syndrome that is becoming a global public health problem with enormous morbidity and mortality. It is a pro-inflammatory and pro-thrombotic ailment characterized by increased platelet activation and alteration of platelet indices. However, the tendency to use platelet indices as predictors of poor glucoregulation is not fully evaluated in our context, and evidence for the role of platelet indices as predictor of poor glycemic status in diabetic patients also limited.*

***Objective:** To assess platelet indices and to determine the predictive value of PLT indices for poor glucoregulation in type 2 diabetic patients at Bishoftu General Hospital, Ethiopia.*

***Methods:** A comparative cross-sectional study was conducted among 261 participants (174 T2DM and 87 non-diabetic controls) from June 15 to Aug 12, 2022, and the systematic random sampling technique was used to select participants. Data were collected using structured questionnaires, physical measurements, checklists, and laboratory tests. Platelet parameters and fasting blood glucose levels were determined from blood samples using Sysm ex-XN550 and CobasC311 analyzers, respectively. Hematology analyzer output was checked and participants were also screened for malaria parasites using a prepared blood smear. Collected data were entered into Epi-data 3.1 version and exported to SPSS-25 version for analysis. Chi-square, Mann-Whitney U test, Kruskal-Wallis test, Post hoc test, spearman correlation, and ROC curve were used for analysis. A P-value<0.05 was considered statistically significant.*

***Results:** Our data showed that PDW, MPV, PLCR, and PCT values were significantly increased in diabetic patients than in healthy controls (p<0.001). Moreover, these indices were significantly elevated in poor glycemic control T2DM than in good control T2DM and healthy controls. Significant correlations with anthropometric and clinical variables was also noted. The PDW at a cut-off value 15.75fl with AUC 0.803; MPV at a cut-off value 12.25fl with AUC 0.774; PLCR at a cut-off value 36.3% with AUC 0.775; and PCT at a cut-off value 0.24% with AUC 0.761; have been identified as predictors of poor glycemic in diabetes mellitus patients.*

***Conclusions and Recommendation*:** *A significant increase in the platelet indices in diabetic patients and their role in predicting poor glycemic in diabetes have been observed. Therefore, regular screening and platelet indices profile checks are recommended during DM follow-up.*

***Keywords:** Platelets indices, Type2 diabetes mellitus, Adult, Ethiopia*

ACKNOWLEDGEMENT

I would like to thank Wolkite University for sponsoring my education. I would also like to thank Jimma University, School of Medical Laboratory Science for giving me this opportunity.

I would like to express my heartfelt gratitude to my advisors Mr. Girum Tesfaye (MSc, Assistant Professor, Ph.D. candidate) and Mr. Gebeyaw Arega(BSc, MSc) for their invaluable constructive direction, guidance and comments through a friendly approach from start to finish of this research paper.

Also, I would like to forward my thank to Dr. Gurmessa Fikru(Medical Director), Dr. Addisu Balayi(internal medicine specialist), Mr. Goshime Worku(Medical laboratory department head) and Mr. Lemma Raagoo(senior Laboratory Technologist) for their clinical advice and permission to use materials and equipment, and all data collectors and others staffs who assist me with data collection and laboratory testing during this study.

Finally, I would like to thank the participants for their sincere participation in this study, those individuals in supported me by providing reagents.

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ABBREVIATIONS AND ACRONYMS

AIDS	Acquired Immune Deficiency Syndrome
AGEs	Advanced Glycation end Product
ADA	American Diabetes Association
ART	Ant-retrovirus treatment
BP	Blood Pressure
BMI	Body Mass Index
CBC	Complete Blood Count
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure
DM	Diabetes Mellitus
K2-EDTA	Dipotassium Ethylene-Di-amine Tetra-acetic Acid
EMLA	Ethiopian Medical Laboratory Association
FBG	Fasting Blood Glucose
GP	Glycoprotein
HC	Hip-Circumference
HIV	Human Immune Virus
IDF	International Diabetes Federation
IQR	Interquartile Range
IL-6	Interleukin-6
MPV	Mean Platelets Volume
NO	Nitric Oxide
ORS	Oromia Regional State
OHA	Oral hypoglycemic agents
PCT	Platelets-crit
PDW	Platelets Distribution Width
PLT	Platelets

P-LCR	Platelet Large Cell Ratio
RAGE	Receptor for Advanced Glycated end Product
ROC	Receiver Operating Characteristic curve
SBP	Systolic Blood Pressure
SPSS	Statistical Package for Social Sciences
T2DM	Type two Diabetes Mellitus
TPO	Thrombopoietin hormone
US$	United State Dollar
WBC	White Blood Cell
WC	Waist Circumference
WHO	World Health Organization
WHR	Waist to Hip Ratio

CHAPTER 1: INTRODUCTION

1.1. Background information

Diabetes is a chronic metabolic disorder caused by a variety of etiologies and defined by persistent hyperglycemia with dysfunction of carbohydrate, lipid, and protein metabolism resulting from abnormal insulin secretion, action, or both(1). Its classification are: Type 1 diabetes results from the immune-mediated destruction of beta cells, resulting in insulin deficiency. Type 2 diabetes due to beta-cell dysfunction, causes insulin resistance. Gestational diabetes is an impaired glucose tolerance that occurs during the second and third trimesters of pregnancy(1,2).It is diagnosed through fasting plasma glucose ≥126 mg/dl;Plasma glucose ≥200 mg/dl after 2 hours after a 75g oral glucose tolerance test; HbA1c≥6.5%; and random blood glucose ≥200 mg/dl in the presence of signs and symptoms of diabetes(1).

Diabetes is characterized by range of disorders such as hyperglycemia, metabolic, cellular, organ, and hematological disorders leading to vascular complications(3). It causes cardiovascular complications and damage to blood vessels with increased blood clotting. Platelets are circulating cells within the vascular system that contribute to hemostasis(4).An increased propensity thrombus activation and formation has been observed in patients with diabetes mellitus. Oxidative stress and the function of pro-oxidant enzymes such as NADPH oxidase appeared as the heart of diabetes-associated platelet hyperactivity(4). Pathological thrombosis associated with atherosclerotic plaque rupture is a major cause of morbidity and mortality. Platelets are intimately involved in the initiation and propagation of thrombosis. There is evidence of increased platelet reactivity and baseline activation in patients with type 2 diabetes compared to healthy controls(5).

Hyperglycemia and insulin resistance cause changes in platelet number and activation, and qualitative and/or quantitative changes in coagulation and fibrinolytic factors, leading to the formation of fibrinolytic-resistant thrombi in diabetic patients(6).

In hyperglycemia conditions platelet activation happened through activation of protein kinase C in vivo and non-enzymatic glycation of platelet proteins(7). In response to hyperglycemia, the neutrophil-derived S100 calcium-binding protein A8/A9 (S100A8/A9) interacts with (RAGE) receptors on hepatic Kupffer cells, increasing the production of IL-6. The production of thrombopoietin enhanced by IL-6, which in turn interacts with its cognate receptor c-MPL

on megakaryocytes and bone marrow progenitor cells to promote their expansion and proliferation, resulting in reticulated thrombocytosis(8).

Factors involved in platelet activation in obese DM patient include elevated plasma leptin, IL-1,IL-6,IFN-α, cytosolic calcium levels. Hypertriglyceridemia causes platelet activation by high levels of glycated low-density lipoprotein (LDL),glycated connective tissue that impairs nitric oxide production and increases cytosolic calcium(9).Obesity increases insulin resistance, cause hyperglycemia and affects the procoagulant activity of tissue factor and cause platelet activation (1,7).Body mass index, waist circumference,and waist-to-hip ratio are anthropometric indicators of central obesity(10).

Upregulation of calcium metabolism and upregulation of the p2Y12 signaling pathway also cause platelet anomalies in DM(11,12). Calcium homeostasis compromised by excessive calcium influx via Na/Ca pumps, activation of calcium ATPases, decreased sensitivity to insulin, and increased oxidative stress. Upregulation of platelet adenosine diphosphate P2Y12 receptor signaling was identified in type 2 diabetic platelets. This signaling suppresses c-AMP levels, decreases insulin response,and increases adhesion,aggregation,and pro-coagulant activity(12).

Platelet activation is manifested by increased expression of biomarkers such as CD31,CD36, CD42,49b,CD62P,CD63 and platelet surface glycoproteins such as GPIb and GPIIb/IIIa, which act as receptors for fibronectin, fibrinogen, von Will brand factor and vitronectin, and release chemokines such as CXCL1L4, CXCL4, CXCL5, CXCL7, CXCL12, CXCL16 which are thereby inducing the recruitment of other cells that cause the thrombotic disorders forwarded (13).

Starting with manual methods, fully automated analyzers using principles of impedance, light scattering, fluorescence, and flow cytometry have been identified for platelet counting. The choice of in vitro anticoagulant, measurement technologies, and post-sampling timeframe can influence platelet count and indices measurements(14).In addition to platelet count, there are several methods to measure wheather platelet are activated or not as a factors in atherothrombotic, such as platelet aggregometry, surface p-selectin, activated glycoprotein IIb/IIIa, platelet function analyzer-100, serum thromboxane B2 and urinary 11-dehydrate thromboxane B2.Most of them are time consuming, expensive, and require special training(15).

Platelet indices have recently received renewed attention for their prognostic function in endothelial dysfunction and inflammatory disease in a simple, rapid, and inexpensive way to diagnose, and alert for the onset or progression vascular complications in diabetic patients(16). One way to identify the platelet indices as indicators of diabetes related impairment can be obtained through receiver operating characteristic. In receiver operational characteristics(ROC), the area under curve(AUC), is the primarily used to assess the diagnostic ability of a test to discriminate the true disease state of within the patients(17).

Platelet parameters such as platelet count and PLT indices(PCT,MPV,PDW & P-LCR) are indicators of platelet activation and are obtained from hematology analyzers(18). Mean platel et volume (MPV) is the most commonly studied platelet parameter, indicating an increase platelet diameter and can be used as a marker of production rate and platelet activation. Platelet distribution width (PDW) measures platelet anisocytosis, changes with platelet activation, an d reflects the heterogeneity of platelet morphology, and is obtained at 20% of the relative hei ght of the platelet size distribution curve. The plateletcrit (PCT) is the percentage of platelet v olume occupied in whole blood and platelet large cell ratio(PLCR) is a discriminator of circul ating larger platelets (>12fl), measured by stable discriminator & reported by percentage(18).

1.2. Statement of the problem

Globally, the prevalence of diabetes mellitus increased five-fold from 108 million in 1980 to 537 million in 2021(19,20). About 73 million people in the Middle East and North Africa an d 24 million people in Sub-Saharan Africa have diabetes (20).The IDF estimates that its prev alence will reach 643 million by 2030 and 783 million by 2045(20). As worldwide, Type 2 diabetes is the predominant, accounting for over 90% of cases and more prevalent in low and middle-income countries(1,2). Diabetes is also prevalent in Ethiopia, with a pooled prevalen ce of approximately 6.5% and 3.3% of adults had diabetes(20,21).

About 541 million people of working age are at risk of developing type 2 diabetes. It is one of the world's major public health challenges, affecting individuals,families,and threatening well-being of entire communities(20). Diabetes is more common in urban than rural areas, an dits prevalence is expected to be higher in middle-income countries compared to high-and low-income countries from 2021 to 2045(20).
Global health care costs for diabetes control increased from US$232 billion in 2007 to US$76 0 billion in 2019 and US$966 in 2021, a 316% increase over 15 years(20). Expenditures in su b-Saharan Africa, also estimated at US$ 67.03 billion annually, or US$8,836 per year per person with diabetes, and diabetes-related spending is estimated at US$7 trillion by 2025(22).

Diabetes is a pro-inflammatory and pro-thrombotic disorder characterized by altered Platelet i ndicesand increased platelet reactivity(23). Activated platelets predispose patients to macrova scular complications (CVD, stroke, and arterial disease, and microvascular complications (ne uropathy, nephropathy, retinopathy),making diabetic patients two to three times more likely t o have stroke or heart attack(19,24).Those phenomena increase diabetes morbidity and mortal ity(24). Large platelets are enzymatically and metabolically active, and are rich in pro-thromb otic molecules such as platelet factors 4,p-selectin, serotonin, thromboxane A2, and platelet-derived growth protein(8,25,26).Increased formation larger platelet leads to atherothrombotic complications in diabetes. Atherothrombotic is the leading cause of vascular disease in patients with type 2 diabetes and increases the risk of coronary artery disease, stroke, and peripheral artery disease(25,26).

About 80% of sufferers with diabetes mellitus die due to thrombotic. Of those, 75% of deaths were because of cardiovascular complications, and the rest is because of cerebrovascular activities and peripheral vascular complications. Endothelial abnormalities play a position within side the superior activation of platelets and clotting elements visible in diabetes (27). According to WHO mortality data base collected from 108 countries from 2000 to 2016 showed about 7.1 million deaths were recorded because of diabetes complications. Of those,approximately 26.8% of mortality were due to vascular complications of injured organs such as kidneys (71.1%), peripheral circulation (27.1%), nerves (1.5%), and eyes (0.3%)(28). As review of diabetes in sub-Saharan Africa found report the proportion of patients with diabetic complications ranged from 7% to 63% for retinopathy,27% to 66% for neuropathy, and 10% to 83% for nephropathy(22).

Early prediction of poor blood glucoregulation in DM patients is very important for the management of diabetes patients and for preventing cardiovascular disease, slowing disease progression, and reducing mortality and morbidity(19,20).There are some evidence support the recent views on the effects of oral hypoglycemic agents on platelet counts and indices(29,30). But there is no data from Ethiopia.To diagnosis the glycemic status and complications status in diabetic patients requires the measurement of various parameters at a time and the availability of these tests limited in our context(19). There are methods to know wheather platelet are activited,but most are time-consuming, overpriced, and require distinct training(15).In our setting they are not available almost at all. Studies conducted on the determination of platelet indices in complicated DM patients in Ethiopia, but they didn't used the control group.

Therefore, finding accessible, reliable, and inexpensive predictors from laboratory reports for early prevention and control of diabetes related disorders, especially in developing countries like Ethiopia with constrained assets.Evidences showed an association between platelet indices and diabetes mellitus(4–8,11,12).

Studies conducted on platelet indices in DM came up with controversial conclusion.A significant increase in platelet indices and their potential prediction for poor glycemic,and vascular complications in the diabetic patients have been identified(23,30–41).

However, the trend to use platelet indices as predictor of poor glucoregulation is not well known in Ethiopia. Therefore, this study aimed to assess the platelet indices in type 2 diabetic adult patients in comparison with healthy control, and to determine the predictive value of PLT indices for poor glucoregulation in DM at Bishoftu Hospital, central, Ethiopia, 2022.

1.3. Significance of the study

Scholars and past experiences demonstrate the gradual increase in mortality, morbidity, and economic impact of diabetes. Although not a substitute for the HgbA1c test, the platelet indic es have been proposed as a predictor of hyperglycemia and complications in diabetic patients.

This finding will help diabetic patients to be saved at early-stage from disorders caused because of the poor blood glucose regulation, and save the community from additional costs for follow-up and monitoring. This is because platelet parameters can be obtained as a routine test and are cost-effective compared to other tests such as HgbA1c.

This findings will help attending physicians in identify and develop strategies for the early management of poor blood glucoregulation. Local health care administrators can also benefit from this study which enabled them to rational resource allocation to facilitate diabetic monitoring and follow-up process. Moreover, it provides valuable information to policy makers and health officials for advance protocols and strategies that improve the use of platelet indices in the monitoring and follow-up of diabetes mellitus guidelines.

It will again contribute to the national and international literature on the platelet indices status in DM and their role in prediction of poor glucoregulation within DM patients, especially from the perspective of developing countries. It will provide data for researchers to conduct further longitudinal multicenter, large-scale studies.

CHAPTER 2: LITERATURE REVIEW

It has been shown to increase platelet indices in diabetic patients compared to healthy controls. For instance, the descriptive study by Alhadas et al. conducted in Brazil in 2016 among 100 type 2 diabetic patients and 100 non-diabetic control found that there was increased PCT, MPV, and PDW in diabetic patients compared to control group. Patients with complications also had higher MPV, PCT, and PDW values than those with uncomplicated diabetes. Also, MPV,PDW and PCT were significantly and positively correlated with FBS level(34).

A comparative cross-sectional study conducted in Delhi, India by Jindal *et al.* in 2011 on platelet indices in diabetes mellitus as indicators of diabetic microvascular complications in 75 subjects found that MPV, PDW, and P-LCR were significantly higher in diabetic patients compared with controls, and PDW found to be higher in diabetics with complications than in those without complications (35).

Another comparative cross-sectional study conducted in Egypt in 2020 by Deris Besada et al.**,** among 50 diabetics, 50 diabetics patients with nephropathy, and 50 healthy subjects, revealed that MPV, PDW, and P-LCR were all significantly higher in diabetic patients compared to the control subjects. However, there was no significant difference between the diabetic and control in terms of PLT & PCT. In diabetic with nephropathy MPV was higher than those without(36).

In addition, according to a cross-sectional study conducted by Pujani et al.in Haryana, India in 2018, among 30 healthy controls, 30 DM patients with complications, and 30 DM patients without complications, revealed that platelet indices, MPV, PCT, and PDW were found to be significantly higher in poor glycemic DM when compared to good control DM, but no significant in PLT & PLCR between the groups. In addition PLT count, MPV,PDW,PCT and PLCR were significantly increased in complicated diabetics than without complicated diabetics(37).

A comparative cross-sectional study done by Biadgo *et al.* in 2016, on 296 participants in Gondor entitled hematological indices and their correlation with fasting blood glucose level and anthropometric measurements in type 2 diabetes mellitus patients revealed that mean platelet volume and platelet distribution width were significantly higher in diabetic patients than controls. But platelet count was not significantly difference the between groups. The PLT and MPV with SBP, MPV with FBS, PLT and MPV with duration of illness the correlation was achieved. The PDW, MPV, and platelet count values have not attained any correlation with BMI and WHR(42).

Another comparative cross-sectional study involved of 246 participants was done on white blood cells and platelet profiles of diabetic patients in Gondar, Ethiopia, by Adane *et al.* in 2021, found that platelet count was significantly greater in diabetics compared to non-diabetic controls. Other platelet parameters, such as PDW, MPV, and P-LCR, were insignificantly elevated in diabetics than in controls. Also, PLT count was correlated with duration of DM illness(43).

In contrast to previous studies, some studies reported insignificant differences between diabetes and control groups in terms of some or all platelet indices. For example, a cross-sectional study done by Chen et al. in 2017, with 20128 participants in the Yangzhou district of China on the relationship between type 2 diabetes and platelet indices reported that PDW and PLT counts were not significantly different between the diabetic and non-diabetic groups, but only MPV was significantly increased in the diabetic group. In this study, DM group and non-DM group participants were different in socio-demographic characteristics such as age, sex, educational level, marital, income, and occupational status(44).

Also, a retrospective study conducted by Jabir *et al.* in 2018, in Kerala India, on a total of 238 participants entitled to platelet count and its correlation with blood sugar level in type 2 diabetes mellitus patients concluded that there is no significant difference in platelet parameters such as platelet count, mean platelet volume, platelet distribution width between diabetic and controls(45).Besides, no statistically significant difference in platelet count, MPV, and PDW were recorded in diabetic patients compared to the control group; as a comparative cross-sectional study done by Ali *et al.* from December 2017 to January 2018 on the assessment of the alteration of blood indices in patients with type 2 diabetic Mellitus in Baghdad, Iraq (46).

Furthermore, by Adam *et al.*in 2021, a case-control study conducted in Khartoum, Sudan among 154 participants with hematological characteristics of type 2 diabetes revealed that there is no significant difference in platelet count, MPV, and PDW between diabetics and the healthy control group. According to this study socio-demographic characteristics such as age, gender, place of residence, and occupational level were not significantly different between DM and the control group(47).In addition, according to a case-control study conducted by Akinsegun *et al.* in 2014, on MPV and platelet counts of type 2 diabetes mellitus and non-diabetic Mellitus controls in Lagos Nigeria with 200 participants revealed that there was no statistically

significant difference in platelet counts among diabetics and healthy controls, while MPV was increased insignificantly in diabetics as compared to control group(48)

Several studies have attempted to show platelet indices as indicators of poor blood glucose management in diabetic patients, with vascular complications and are annoyed to show the correlation with some factors that affect platelet indices directly or indirectly. For example, Rajagopal *et al.* in 2018, a cross-sectional study on 450 participants in Tamil Nadu, India found that, the MPV, PDW, and PLCR were significantly greater in poor controlled diabetes than in good controlled diabetics and non-diabetics. Mean platelet volume, PLCR, and PDW had showed significant positive correlation with FBS, whereas PCT and PLT had not correlation with FBS(38).

In a cross-sectional study done by Dwivedi et al, on the Variation of Platelet Indices among Patients with Diabetes Mellitus Attending Tertiary Care Hospital in Belgaum 2018, among 210(53 without complications and 157 with complications) DM patients, the MPV, PDW and PLCR were significantly higher in complicated DM than without complicated DM patients. However, platelet count value was insignificantly higher in DM without complication patients(39).

In a comparative cross-sectional study conducted by Shahzad Ali Jiskani et al. on platelets indices as biomarkers of glycemic control and progression of complications in patients of diabetes mellitus type 2 in 2021 in Pakistan among 35 normal controls, 35 DM type 2 patients without complications and 35 DM type 2 with complications, revealed that MPV, PCT & PLCR values were significantly lower in type 2 diabetes with complications as compared to other groups. But PLT count and PDW were not showed any significant difference between groups(49).

A comparative cross-sectional research conducted in Athens Greece by Dalamaga et al in 2010 among 90 participants showed that the MPV and PDW values were higher in diabetic patients with poor glycosylated hemoglobin and poor fasting glucose controls than in good controls and healthy controls group(40).Also, a cross-sectional study conducted by Atak *et al.* in 2018, in Bolu Turkey on the association of platelet distribution width with type 2 diabetes mellitus, diabetic nephropathy, and neuropathy reported that the PDW was significantly increased in T2DM with nephropathy compared to healthy control subjects, and also in diabetic with nephropathy and neuropathy it was significantly elevated compared to diabetic subjects without

these diabetic complications. Also, PDW was significantly correlated with fasting plasma glucose (41).

However, a retrospective study entitled by the relationship between mean platelet volume with micro-albumin and glycemic control in patients with type2 diabetes mellitus done by Unubol et al., in Aydin Turkey 2012, among 354 patients revealed that MPV was not significant in poor glycemic control diabetic patients(50).According to a comparative cross-sectional study done in Ankara Turkey 2018, by Kizilgul et al. among 135 type 2 diabetes with poor glucose control and 121 healthy subjects found that PDW was higher in the DM group and was positively correlated with age, DBP, FBS($p<0.05$). However, platelet count and MPV were not showed any significant difference between diabetics and controls, and no correlation was seen with age, DBP, SBP BMI, and WC(23).

A study conducted by Zoungas et al.in Australia in 2014, entitled by the association between age, duration of DM illness and major macrovascular events, all-cause death, and major microvascular events were examined among 11,140 patients with type 2 diabetes, showed that patients with type 2 diabetes, age at diagnosis and duration of DM were independently associated with macrovascular events and death whereas the only duration of DM is independently associated with microvascular events(51).

In terms of the anti-diabetes and platelet indices relationship, a comparative cross-sectional study done by Abker *et al* in 2021 in Sudan, among 96 diabetic patients and 50 healthy controls revealed that both types of drugs reduced platelet indices except platelet count when compared to the control. Platelet indices were reduced among an oral hypoglycemic study group than a non-diabetic population so the oral hypoglycemic have shown a good prognostic effect on Pro-thrombotic state and accelerated atherosclerosis of DM but this effect was much more reduced among those using Metformin than patients taking Glimepiride as an oral hypoglycemic drug(30).

A case-control study conducted in Pune, India, by Joshi *et al.* in 2019, involved 120 participants report showed MPV and PDW values were higher in cases than controls. Platelet distribution width showed a significant positive correlated with FBS levels in cases. The ROC analysis for MPV and PDW as predictors of glycaemia level, and the optimum cut-off value of MPV was 9.5fl with a sensitivity of 78.33%, specificity of 70%, and a positive predictive value of 72.30%, while the optimum cut off value of PDW was 15fl with a sensitivity of 81.66%, specificity of

91.66%, and a positive predictive value of 90.74%. The study shows a strong association between PDW and HbA1c and it was used as an indicator of impending vascular events(31).

Also, a cross-sectional study done by Sadikuj J et al. in 2017, in Bangladesh on the association of mean platelet volume and platelet distribution width with HbgA1c among a total of 87 T2DM patients showed that MPV and PDW are significantly higher in diabetes patients with poor glycemic control. In this study, ROC analysis of MPV and PDW showed moderate quality indicator of blood glucose regulation deteriorations at best cutoff value 9.55fl with a sensitivit y of 85.19%,specificity of 30.30%, PPV of 58.23%,NPV of 12.5%,and at cut-off 14.55fl with a sensitivity of 45.77%,specificity of 67.86%,PPV of 67.86%,NPV of 37.25%, respectively(32).

In addition, another cross-sectional study conducted on 106 T2DM participants done by Kadic et al., in Bosnia and Herzegovina 2015, report that MPV was significantly higher in poor glycemic diabetes when compared with good controlled diabetic patients. Also, this finding was reported that MPV was positively and significantly correlated with FBS and ROC analysis indicated that MPV was the main predictor for poor glucoregulation with AUC 0.726, at a cut-off value 9.55fl with a sensitivity of 82% and specificity of 54.5%(33).

In general, the above literature review showed platelet indices changes in patients with diabetes. Studies conducted in the different areas on platelet indices of type 2 diabetic patients came up with dissimilar results. Several studies have shown statistically significant increases in the platelet indices in diabetics compared with healthy controls(34–37,42,43). Other studies have reached conflicting conclusions(44–48).

Additionally, some studies show that the platelet indices as a predictor of poor glycemic, vascular complications, and their correlations with some factors(23,31–33,38–42,51). However, some studies have provided conflicting reports(23,49,50).The Platelet indices have recently received renewed attention due to their prognostic function in endothelial dysfunction and inflammatory conditions in diabetic patients in a simple, rapid, and inexpensive way(16). Therefore, this study aimed to assess platelet indices in T2DM adult patient in comparison with healthy controls and to determine the predictive value of PLT indices for poor glucoregulation in DM at Bishoftu General Hospital, central Ethiopia, 2022.

2.1. Conceptual framework

This conceptual framework was proposed after several kinds of literature were reviewed. It is an attempt to show an association between study variables.

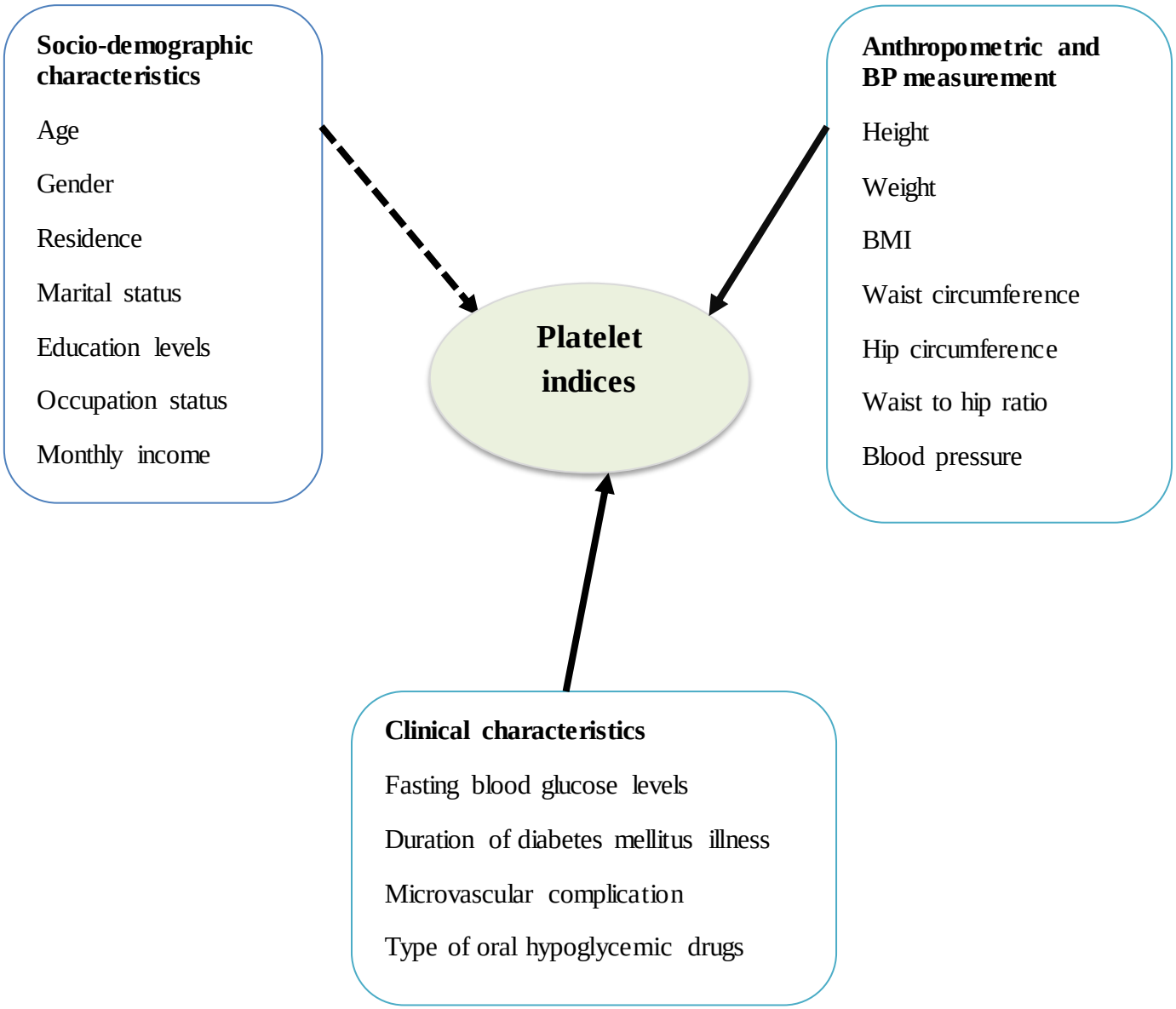

Figure 1: Conceptual frame work for the study conducted on assessment of platelet indices among type 2 diabetic adult patients at Bishoftu Hospital, 2022.

--> The broken line shows the association between the distal predictor variables and the outcome variable.

→ The solid line indicates the association between the proximal predictor variables and the outcome variable.

CHAPTER 3: OBJECTIVES

3.1. General Objective

To assess platelet indices in type 2 diabetic patients in comparisons with healthy controls and to determine the predictive value of PLT indices for poor glucoregulation in type 2 diabetic patients at Bishoftu General Hospital, central Ethiopia, from June 15 to August 12, 2022.

3.2. Specific objectives

- To compare platelet indices between type 2 diabetes mellitus adult patients and control groups at Bishoftu General Hospital during the study period.
- To compare platelet indices between poor glycemic control type 2 diabetes mellitus adult patients, good glycemic control type 2 diabetes mellitus adult patients, and healthy controls at Bishoftu General Hospital during the study period.
- To determine the correlations of platelet indices with anthropometric and clinical variables in type 2 diabetes mellitus adult patients at Bishoftu General Hospital during the study period.
- To determine the predictive ability of the platelet indices for poor glycemic control of type 2 diabetes mellitus adult patients at Bishoftu Hospital during the study period.

CHAPTER 4: METHODS AND MATERIALS

4.1. Study area and Period

This study was conducted at Bishoftu General Hospital from June 15 to August 12, 2022. Bishoftu General Hospital is one of the hospitals found in Oromia Regional State (ORS) and is found in Bishoftu town 47 kilometers apart, southeast of Addis Ababa. The town lies at an altitude of 1950 meters above sea level, at 9^0N latitude and 40^0E longitude(52). There are two public hospitals, five health centers, two private hospitals, and ten private clinics, serving people in the catchment from preventive and basic curative to advanced medical services.

Bishoftu General Hospital was established in 1948 and currently serves approximately 1.2 million people with a variety of services(53). The hospital offers chronic care, emergency ser vices, ART services, surgical, dental, medical services, ophthalmology, pediatrics, gynecolog y & obstetric, radiology,physiotherapy,Pathology services, pharmacy and laboratory services, and others. It is giving follow-up services that include (ophthalmology services, physical examinations, laboratory services such as determination of FBG level, Urea and creatinine m easurement, urine ketone screening, and rarely CBC services) for more than 1,900 diabetic pa tients, of which 1260 T2DM and 640 T1DM. This study was conducted in central laboratory Bishoftu hospital providing hematology and immunohematology,parasitology, microbiology, clinical chemistry, and serology services.

4.2 Study Design

A hospital-based comparative cross-sectional study was conducted.

4.3. Population

4.3.1. Source population

All adult patients already diagnosed with type 2 diabetes mellitus and who are on follow-up in the chronic care clinic of Bishoftu Hospital as cases and, as controls, all healthy non-diabetic individuals who came to Bishoftu Hospital for a medical checkup, and patient attendants were source population of this study.

4.3.2. Study population

All adults with type 2 diabetes attending the chronic care clinic at Bishoftu General Hospital voluntarily gave written informed consent and met eligibility criteria as cases and as controls matched by age and sex non-diabetic individuals who came to Bishoftu General

Hospital for a medical checkup, and patient attendants during the study period were enrolled as study populations.

4.4. Eligibility criteria

4.4.1. Inclusion criteria

All patients with type 2 diabetes who had followed consecutively for more than three months in the chronic clinic unit, aged between 18 to 65 years, and as control group matched by age and sex non-diabetic individuals who came to Bishoftu hospital for a medical checkup and patient attendants during the study period were eligible for this study.

4.4.2. Exclusion criteria

Based on information acquired from a medical record, from an interview of study participants, and information gotten through physicians' communication, the type 2 diabetic patients with a history of chronic diseases like renal failure, liver, hematological malignancy, patients with a history of infectious disease such as HIV/AIDS, HBV, HCV, malarial infection, asthmatic, rheumatoid arthritis, patients who were severely ill, anticoagulant therapy, on antiplatelet drugs, pregnant mothers, smokers, alcoholics, and anemic patients were excluded from this study. Also the health condition of the control group was evaluated, and individuals who had a history of malarial infection in the past two months, whose C-reactive Ab test was positive, smokers, and alcoholics were excluded from the study.

4.5 Study Variables

4.5.1 Dependent variables

- Platelet indices

4.5.2Independent variables

- Socio-demographic variables such as: Sex, age, residence, educational level, marital status, occupational status, and monthly income.
- Anthropometric and Blood Pressure measurements such as: Height, weight, BMI**,** waist circumference, hip circumference, WHR, and Blood pressure.
- Clinical characteristics include: fasting blood glucose level of last two months, duration of diabetes mellitus illness, microvascular complication, and type of oral hypoglycemic drugs.

4.6. Sample size and sampling technique

4.6.1 Sample Size Determination

The sample size was determined based on the two population mean formula using G-power software version 3.1. During sample size determination the 95% CI(2-tailed,α=0.05),80% power, a ratio of the control to case 1:1, a computed effect size (d) was 0.45, and a non-respondents rate of 10% was considered. The mean and standard deviation of MPV for DM patients and control groups were taken from a study done at Gondar(42),{10.4±1.1} for type 2 diabetes, and {9.9±1.1} for controls. To increase accuracy, the rule of thumb of Van Voorhis and Morgan(54) was used, and cases were twice to the controls, and determined sample size for T2DM was 174,and for control group was 87,and we got a total of 261 participants for this study.

4.6.2 Sampling Technique

Study participants were selected using a systematic random sampling method based on the order of follow-up visits attended. The sampling interval (the k^{th} value) was obtained by divid ing the estimated total number of the source population by the sample size (approximately 7). The hospital report of last quarter estimated the number of people with type 2 diabetes at follow-up were 1260, and the calculated sample size for the cases was 174. Then, the k^{th} value =1260/174=7, and the first participant was selected by lottery method through writing the medical record numbers of the first seven participants on a separate piece of paper, and thereby individual who came on second number was selected as first participants. Subsequently patients with type 2 diabetes were then interviewed face-to-face in between the service at every seven intervals of order of visit.

4.7. Data collection procedure

4.7.1 Study participants recruitment

A total of 261 study participants from two groups were recruited for this study. The first group included 174 T2DM patients and the second group was 87 non-diabetic healthy controls. Based on the ADA-2017(55) criteria, the 174 T2DM study subjects were sub-grouped into 87 poor glycemic control type 2 diabetes mellitus group (whose FBG level >130mg/dl with the conjunction of their worse clinical history and the remaining 87 were good glycemic control type 2 diabetic patients (whose FBG level was 80mg/dl to 130mg/dl and their clinical history was also good. Based on information from history medical records of patients, poor glycemic control type 2 diabetic patients were further categorized into patients with complications and

without complications. Structured questionnaires for socio-demographic, anthropometric, and BP measurements, checklists, and laboratory tests were used to collect the data for this study.

4.7.2 Socio-Demographic data collections procedure

The socio-demographic data were obtained from study participants through a structured quest ionnaire via face-to-face interviews followed by Covid-19 protection and prevention protocol.

4.7.3 Clinical data, Blood pressure, and Anthropometric data collections procedure

Checklists were used to extract clinical characteristics such as duration of DM illness, presence or absence of microvascular complications, type of oral hypoglycemic drugs, and fasting blood glucose levels in the last two months from patients' history on medical records. Diabetic patients fasting blood glucose readings for at least three months including the current reading was used for computing the average blood glucose level.

After the participants took a rest for more than five minutes, blood pressure (BP) measurement was taken from the upper arm of the left hand at the heart level by sphygmomanometer and stethoscope analogs twice simultaneously within two minutes intervals, and reported by millimeter mercury (mmHg). The stadiometer and weighing scale was used for the measurement of height (to the nearest 0.1 centimeters without shoes), weight (to the nearest 0.1 kg) without shoes, and wearing very light clothing; then BMI was computed by dividing the weight in kilograms for height in meters squared.

Waist circumference (WC) was measured with a non-stretchable tape at the midway between the least palpable inferior margin of the rib and the iliac crest in the participant's normal exhaled state. The hip circumference (HC) was also measured using the same tape around the widest portion of the buttocks, and then the waist-to-hip ratio (WHR) was computed by dividing WC in centimeters by HC. All anthropometric measurements were performed by clinical nurses according to the WHO anthropometric protocol. Anthropometric and blood pressure measurements were performed twice and the mean values were used for analysis.

4.7.4 Blood Sample collection Procedure

After an interview, record review, and anthropometric and blood pressure measurements by a clinical nurse, study subjects were sent to a central laboratory, and then the vein of antecubital fossa of the forearm was disinfected with 70% alcohol, and a tourniquet was applied as needed, then after the required amount of blood had been collected. A total of 5ml venous blood sample was collected from each T2DM patients by trained laboratory technologist in two different

tubes; 3ml of blood sample was taken into a K2-EDTA test tube for platelet parameter determination, blood smear preparation, and 2ml into standard serum separator test tube without anticoagulant for serum glucose analysis.

The blood sample collected in a serum separator tube stayed for 15 minutes at room temperature; then serum was separated by centrifugation at 3600rpm for 3 minutes using a Sorvall-ST-16 centrifuge, and serum was used for glucose determination. With the same procedure and precaution, 3ml of venous blood was collected into a K2-EDTA test tube from each control participants for determination of PLT parameters, blood smear preparation and C-reactive protein Ab test (**IV**).

4.7.5 Sample analysis procedure

Fasting blood glucose was determined from the serum sample prepared from an organ testtub e by CobasC311(Roche system, Germany)following the photometric analysis principle and detecting glucose level in serum spectrophotometrically at 340nm(56). A five differential aut omated hematological analyzer Sysmex XN550(Sysmex, Germany)which is working by the c ombination of hydro-dynamically focused impedance measurement for RBC and platelet cou nt, flow cytometer method for WBC differential count and photometric principle for hemoglo bin determination was used(57).

Thin blood film was prepared, labeled, air-dried, and then stained by Wright stain to recheck platelet count output of hematology machine & to evaluate morphological arrangement, and it was also used to screened participants for malaria. Fasting blood glucose level, PLT count and PLT indices (MPV, PDW, PCT, and P-LCR) were analyzed at Bishoftu General Hospital central laboratory, and the result of each study participant was printed and attached to request paper and recorded using laboratory result registration form **(IV).**

4.8. Operational definitions

Adult: a study participant aged 18 to 65 years.

Controls group: are non-diabetic individuals who came to Bishoftu hospital for medical checkup for the purpose of job recruitment, driving licensing and patient attendants.

Anthropometric variables: Are measurements these includes in this study are height, weight ,body mass index, waist circumference, hip circumference, and waist-to-hip ratio.

Body mass index: WHO-defined for both sex and adults: BMI <18.5 kg/m^2 indicates underweight, a BMI 18.5-24.9 kg/m^2 indicates normal weight, a BMI 25.0-29.9 kg/m^2 indicates overweight, and a BMI ≥ 30 kg/m^2 indicates obese (10).

Clinical variables: According to this study clinical variable are type of oral hypoglycemic dr ugs, FBS level, duration of DM illness, and status of microvascular complications.

Glycemic control: The ADA recommends measuring HgbAc1 to determine glycemic control levels. However, if the HgbA1c test is unaffordable and unavailable, the mean fasting blood glucose level at three consecutive visits can be considered to assess glycemic control in DM patients(55). Accordingly, **Good glycemic control**: Mean FBG level over three months is 80–130 mg/dl. **Poor glycemic control**: three months mean of FBG level > 130mg/dl.

Microvascular complications: Of diabetes are those long-term complication that affects small blood vessels. Typically include diabetic retinopathy, diabetic neuropathy, and diabetic nephropathy(35).

Platelet indices Are part of CBC parameters which include (PLT count(150-450x10^3/µl),MP V(9-11fl),PCT(0.17-0.23%),PLCR(14-35.0%),PDW(9-14.0fl).

Waist circumference(WC): In adult subjects, WC measurements >94cm in males and >80cm in females suggest central obesity (10).

Waist-to-Hip ratio(WHR): A WHR >0.90 in adult men and >0.85 in adult women indicates abdominal obesity (10).

Thrombocytopenia: Defined as a platelet count <150x10^3/µl(43).

Thrombocytosis: A condition in which platelet count is >450x10^3/µl(43).

4.9. Data quality management

To ensure data quality, questionnaires and informed consent forms written in English were translated into the local language Afaan Oromo and Amharic, and retranslated into English for accuracy and consistency. Before the actual data collection, a pretest was done on 5% of the total sample size (13 subjects met the eligibility criteria) at Mojo Hospital, which is located in East Shewa, Mojo town. Half-day training was given for four data collectors (two clinical nurses and two laboratory technologists), on study objectives, data collection procedures, and confidentiality of information in order to reduce technical and observation bias.

To maintain the quality of the socio-demographic, anthropometric, and clinical data, daily completeness and consistency was checked by on-site supervision of the data collectors during the study period. A code was used to maintain the confidentiality of study participants' test results, and records were kept in a secret, inaccessible place. Feedback and corrections were provided as needed based on the daily data collection process.

The manufacturer's instructions and standard operating procedures were followed during specimen collection, CBC analysis, fasting blood glucose determination, and blood film preparation and examination to maintain the quality of laboratory data. Thus, to avoid post-collection hemolysis, the blood sample was dispensed to the walls of a K2-EDTA test tube, gently inverted 8-10 times to mix properly, and platelet counts & indices were determined. All platelet count below, above, or normal to the reference range, and their arrangement was observed under microscope by using wright-stained thin blood film.

Acceptance criteria such as hemolysis, clotting, adequate sample volume, and collection time were checked. To avoid mix-up after collection, labeling was done on the sample holder and t he request paper with the same identification code.Based on the Hospital laboratory protocol, low, normal, and high control materials were used for the hematology analyzer. Normal and p athological control materials were performed for the Cobasc311 analyzer, when measuring glucose respectively. Background readings were obtained daily to reduce background carryover effects. Reagent expiration dates were checked before the analysis of patient samples. All laboratory assays were analyzed within 2 hours of sample collection, all laboratory test results were recorded, and reported and samples were properly managed.

4.10. Statistical data analysis and Interpretations

Collected data were checked for completeness and consistency, entered into Epi-Data version 3.1 (Epi-Data Association, Denmark), and analyzed using Statistical Package for Social Sciences (SPSS) software version 25 (IBM SPSS statistics, USA).Histograms and the Kolmogorov-Smirnov test were used to check the normality of the data distribution. Results for categorical variables were reported in frequency and percentage. For these categorical variables, statistical differences were determined by the chi-square test.

Continuous parameters were not normally distributed in the goodness-of-fit model test. Therefore, descriptive analyses were reported as median (interquartile range) or median

(IQR). Mann-Whitney U test and Kruskal-Wallis analyzes were used to compare platelet indices between groups. Bonferroni's test was used as a post-hoc to compare the values of platelet indices between the three groups. Correlations between the platelets indices and anthropometrics and clinical variables were assessed using bivariate Spearman's rank correlation coefficients. Receiver operating characteristic curves were constructed to determine the sensitivity, specificity, cutoff value, area under curve, PPV, and NPV in distinguishing between poor and good glycemic control in diabetic patients. A P-value<0.05 was considered statistically significant.

4.11. Ethical consideration

Approved ethical clearance was obtained from the Institutional Review Board of the Jimma University Institute of Health under Ref.No.IRB84412/2022.A supplementary letter,Ref.No. BEFO10339/2022, was also obtained from the Oromia Regional Office, Department of Research Review Board, and submitted to Bishoftu General Hospital administration office and was directed to medical laboratory head and chronic clinic head offices. It was then sent to both headquarters along with a letter of support from the School of Medical Laboratory Sciences, Jimma University.

After obtaining permission from the Hospital administrator, the head of the Chronic Clinic care unit, and head of medical laboratory, a clear explanation of the study's objectives, procedures, benefits, possible risks, and the participant's right to voluntarily participate, written informed consent was obtained from each study subject. Codes were used instead of participants' names to protect the confidentiality of the data obtained, and unauthorized access to the data collected was prohibited. For participants whose platelet count and PLT indices were abnormal physicians were communicated for appropriate treat of these patients.

4.12. Dissemination plan

The findings of this study will be presented to the School of Medical Laboratory Sciences, Institute of Health, Jimma University. Then, it will be disseminated to Oromia Regional Health Bureau and Bishoftu General Hospital. The research abstract will be submitted to local associations such as EMLA, and the findings will be present at continuing medical education events organized by the associations. Attempts will be made to present the findings at various scientific conferences and publish the results of this research in international or national peer-reviewed reputable journals.

CHAPTER FIVE: RESULTS

5.1 Socio-demographic characteristics of the study participants

Sociodemographic data showed that the type 2 diabetic patients and healthy control study groups did not differ significantly in terms of age and sex ($p>0.05$). The median (IQR) of age in T2DM was 33(28-39) and in healthy controls was 31(27-38) ($p=0.322$).Most of the study participants were males, 139(79.9%) with type 2 diabetes and 66(75.9%) with healthy controls **(**Table 1).

Approximately 147(84.5%) of T2DM and 68(78.2%) controls were from urban residence. The majority of 66(37.9%) type 2 diabetic patients and 50(57.5%) healthy controls had secondary educational levels. At the occupational level, 65(37.4%) of type 2 diabetic patients were government employed and 37(42.5%) of healthy controls were unemployed. Regarding marital status, 75(43.1%) of type 2 diabetes patients and 35(40.2%) of the control group were married. Approximately 95(54.6%) with T2DM had medium monthly income, whereas 40(45.9%) of healthy controls had low monthly income (Table 1).

Table **1:** Socio-demographic characteristics of the study participants at Bishoftu General Hospital, central Ethiopia, from June 15 to August 12, 2022.

Variables	Categories	T2DM patients	Healthy controls	p-value
Age	Media(IQR)	33(28-39)	31(27-38)	0.322
Sex	M n(%)	139(79.9%)	66(75.9%)	0.46
	F n(%)	35(20.1%)	21(24.1%)	
Residence	Urban n(%)	147(84.5%)	68(78.2%)	0.21
	Rural n(%)	27(15.5%)	19(21.8%)	
Educational level	Unable to write & read n(%)	8(4.6%)	2(2.3%)	0.04
	Can write and read n(%)	11(6.3%)	2(2.3%)	
	Primary school n(%)	25(14.4%)	9(10.3%)	
	Secondary school n(%)	66(37.9%)	50(57.5%)	
	Higher education n(%)	64(36.8%)	24(27.6%)	
Occupation status	Unemployed n(%)	37(21.3%)	37(42.5%)	0.004
	Merchant n(%)	47(27.0%)	20(23%)	
	Farmer n(%)	25(14.4%)	7(8%)	
	Gov't employee n(%)	65(37.4%)	23(26.4%)	
Marital status	Single n(%)	59(33.9%)	33(37.9%)	0.94
	Married n(%)	75(43.1%)	35(40.2%)	
	Divorced n(%)	29(16.7%)	14(16.1%)	
	Widowed n(%)	11(6.3%)	5(5.7%)	
Monthly Income	<1500ETB n(%)	54(31.0%)	40(45.9%)	0.049
	1500-6000ETB n(%)	95(54.6%)	35(40.2%)	
	>6000ETB n(%)	25(14.4%)	12(13.8%)	

Key Note: IQR-Interquartile range, T2DM-type 2 diabetes mellitus, ETB-Ethiopian birr, M-male, F-female

5.2 The anthropometric and clinical characteristics of study participants

Regarding the anthropometric and clinical characteristics of the study participants, there were statistically significant differences between groups concerning BMI, WHR, SBP, and DBP at (P<0.001) and for WC (p=0.017). The median (IQR) of FBG was 131.2(114.7-150) mg/dl, and the duration of DM illness was 6.0(3.0-10.0) years in T2DM patients. Among type 2 diabetes with uncontrolled glycemic control, about 45(51.7%) were experienced at least one type of microvascular complication, and about 89(51.1%) of diabetes patients receive oral hypoglycemic therapy with metformin treatment (**Table** 2).

Table **2:** Anthropometric and clinical characteristics distribution of study participants at Bishoftu General Hospital, central Ethiopia, from June 15 to August 12, 2022.

Variables	Categories		T2DM patients	Healthy control	P-value
BMI(Kg/m^2)	Media(IQR)		23.2(20.6-25.3)	20.3(19.1-22.03)	<0.001
WC(cm)	Media(IQR)		90(87-97)	89(86-92)	0.017
WHR	Media(IQR)		0.91(0.86-0.98)	0.87(0.83-0.92)	<0.001
SBP(mmHg)	Median(IQR)		141(133-150)	131(126-135)	<0.001
DBP(mmHg)	Media(IQR)		101(94.0-109.0)	90(87.0-92.0)	<0.001
FBGlevel(mg/dl)	Media(IQR)		131.2(114.7-150)	—	—
Duration of DM illness(years)	Media(IQR)		6.0(3.0-10.0)	—	—
Presence of microvascular complications at least one type	Poor glycemic T2DM(87)	Yes n(%)	45(51.7%)	—	—
		No n(%)	42(48.3%)		
	Good glycemicT2DM(87)	Yes n(%)	0(0%)	—	
		No n(%)	87(100%)		
Current Oral hypoglycemic therapy used by DM patients	Glibenclamide n(%)		41(23.60%)	—	—
	Metformin n(%)		89(51.1%)	—	
	Metformin+Glibenclamide n(%)		44(25.3%)	—	

Key Note: BMI-body mass index, WC-waist circumference, WHR-waist to hip ratio, SBP-systolic blood pressure, DBP-diastolic blood pressure, FBS-fasting blood sugar, DM-diabetes Mellitus.

5.3 platelet parameters among study participants

5.3.1 Morphological Analysis Report

Microscopic examination of the peripheral blood film was done for all participants to check hematology automation output result and morphological arrangement. Accordingly, about 102(58.6%) type 2 diabetes mellitus individuals' platelet morphology picture were Giant in size, while, 61(35.1%) type 2 diabetes mellitus patient platelet morphology picture were larger platelet in size. Normal platelet size picture were observed in among 11(6.3%) type 2 diabetes mellitus patient blood smear. In healthy control group, only 3(3.5%) of individuals platelet picture were larger in size feature. In all participants morphological blood picture the clumping or satellitism feature was not observed.

5.3.2 Frequency of platelet abnormality among diabetic patients

According to this study, thirty (30) of our study participants experienced thrombocytopenia which accounts for 17.2%, while, nine (9)of our study participants have appeared with throm bocytosis, these accounts for 5.2% (Figure 2)

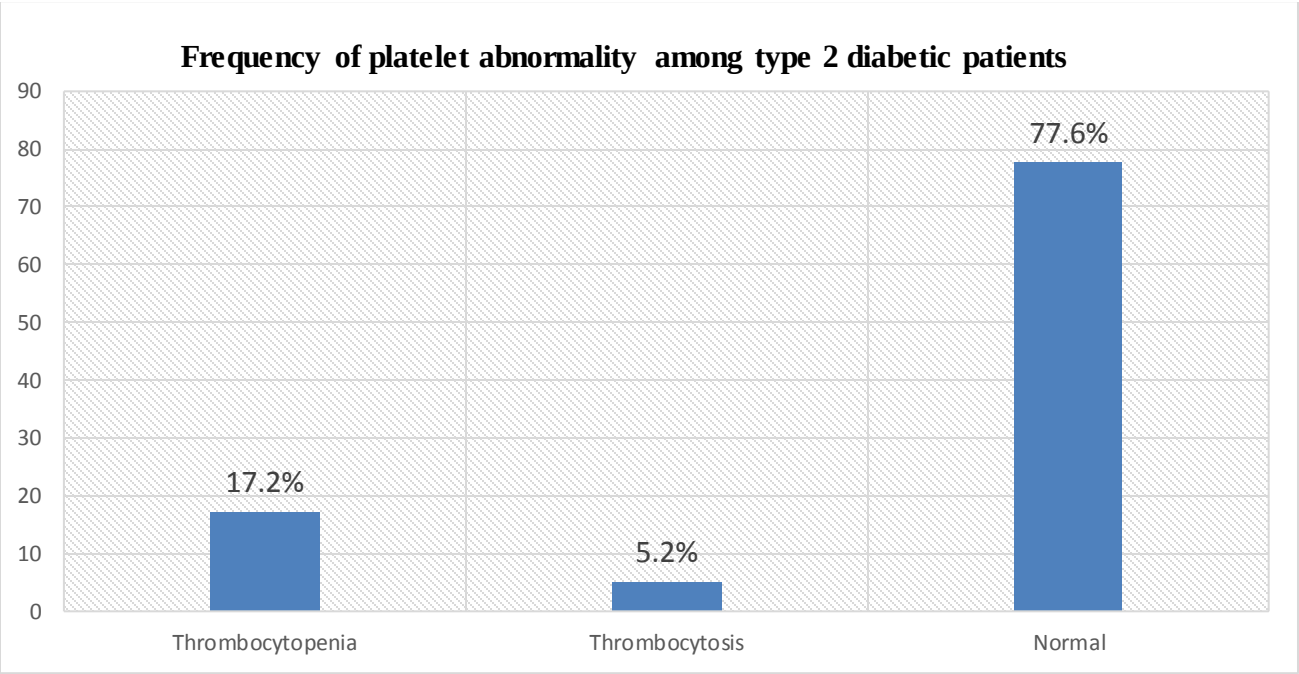

Figure **2:** Frequency of platelet abnormality in type 2 diabetic patients attending Bishoftu Hospital from June 15 to August 12, 2022.

5.3.3 Comparisons of platelet indices between diabetic patients and healthy controls

The median (IQR) of the PLT was 198(160-291) $x10^3$/µl in T2DM, and 208(170-232) $x10^3$/µl in healthy controls (p=0.269).On the other hand, PDW, MPV, PLCR values were significantly increased in diabetic patients than in healthy controls with p<0.001); whereas PCT with (p=0.001) **(**Table 3**).**

Table **3:** Comparisons of platelet indices between diabetic patients and healthy control group at Bishoftu Hospital, central Ethiopia, from June to August 2022**.**

Platelet parameters	Diabetes mellitus group Media(IQR)	Healthy control group Media(IQR)	P-value
Platelet count(10^3/µl)	198(160-291)	208(170-232)	0.269
PDW(fL)	13.7(11.8-20.1)	11.1(10.1-13.1)	<0.001
MPV(fL)	11.1(10.3-13.2)	9.9(9.6-10.9)	<0.001
PLCR (%)	33.2(27.3-46.2)	24.5(21.2-31.8)	<0.001
PCT (%)	0.24(0.2-0.32)	0.23(0.19-0.26)	0.001

Key Note: PDW-platelet distribution width, MPV-mean platelet volume, PLCR-platelet large cell ratio, PCT-plateletcrit

5.3.4 Comparisons of platelet indices across poor glycemic controlled T2DM, good controlled T2DM, and healthy controls by Kruskal Wallis test and Post hoc test.

There was no significant difference in platelet counts between the three groups of data according to the Kruskal-Wallis test (p=0.512). However, the values of platelet indices such as PDW, MPV, PLCR, and PCT were significantly different between T2DM with poor glycemic control, T2DM with good glycemic control, and healthy control (p<0.001)(Table 4).

The Bonferroni test was used as a post hoc test for these platelet indices with the level of significance set at *P*<0.05 in the Kruskal-Wallis test to evaluate the difference between the entire groups. The Bonferroni pairwise comparison test showed no statistically significant difference between good glycemic controlled T2DM and healthy controls group in terms of PCT (p=1.000).

In contrast, other platelet indices such as PDW (p=0.009), MPV (0.01), and PLCR (0.009) were elevated in type 2 diabetics with good glycemic control compared to healthy controls. In addition, platelet indices such as PDW, MPV, PLCR, and PCT values were significantly increased in type 2 diabetic patients with poor glycemic-controlled compared to good-controlled type 2 diabetes and healthy controls (p<0.001)(Table 4).

Table 4: Kruskal Wallis and Bonferroni pairwise comparisons of platelet indices between poor glycemic controls T2DM, good glycemic control T2DM, and healthy control at Bishoftu Hospital, central Ethiopia, from June to August 2022.

Platelet indices	Poor control T2DM Media(IQR)	Good control T2DM Media(IQR)	Healthy control Media(IQR)	Kruskal Wallis test	Post hoc test (Bonferroni test)		
				p-value	P*-value	P**-value	P***-value
PLT count (10^3/µl)	188(159-350)	210(162-251)	208(170-232)	0.512	—	—	—
PDW(fL)	19.1(13.3-24.2)	12.4(11.6-13.7)	11.1(10.1-13.1)	<0.001	0.009	<0.001	<0.001
MPV(fL)	12.8(10.8-14.8)	10.7(10-11.2)	9.9(9.6-10.9)	<0.001	0.01	<0.001	<0.001
PLCR (%)	44.4(31.6-55.2)	30(25.8-34)	24.5(21.2-31.8)	<0.001	0.009	<0.001	<0.001
PCT (%)	0.27(0.22-0.38)	0.23(0.2-0.28)	0.23(0.19-0.26)	<0.001	1.000	0.001	<0.001

Key Note: The p-value was significant at *p-value*<0.05, whereas:-

***P**-stands for comparison of good glycemic controlled T2DM and healthy control group.

****P**-stands for comparison of good glycemic controlled T2DM and poor controlled T2DM.

*****P**-stands for comparison of healthy control and poor controlled T2DM group

5.3.5 Comparisons of platelet indices across microvascular complicated T2DM and uncomplicated T2DM.

Among T2DM patients with poor blood glucose levels, approximately 45 participants experienced at least one type of microvascular complication. However, 42 had no complications and the platelet indices were assessed among these groups. Thus, the type 2 diabetic patients with microvascular complications had significantly elevated median (IQR) values of PDW, MPV, PLCR compared to type 2 diabetic patients without complications ($p<0.001$).On the other hand, in the Mann-Whitney comparison test, the median (IQR) of PLT count 169(142-187), and the PCT values 0.24(0.20-0.28) were significantly decreased in type 2 diabetic patients with complications when compared with those uncomplicated type 2 diabetic patients who had 334(193-422) and 0.38(0.27-0.42) values for PLT count and PCT,respectively ($p<0.001$)(**Table** 5).

Table **5:** Comparisons of platelet indices between microvascular complicated T2DM and without complicated T2DM patients at Bishoftu Hospital, central Ethiopia, from June to August 2022.

Platelet indices	Microvascular complicated patients, Media(IQR)(n=45)	Uncomplicated diabetic patients, Media(IQR)(n=42)	P-value
Platelet count(10^3/µl)	169(142-187)	334(193-422)	<0.001
PDW(fL)	23.1(19.1-24.8)	14.7(12-18.1)	<0.001
MPV(fL)	13.9(12.9-15.1)	11.4(10.1-12.7)	<0.001
PLCR (%)	52.8(47.0-57.0)	33.3(26.2-42.8)	<0.001
PCT (%)	0.24(0.20-0.28)	0.38(0.27-0.42)	<0.001

5.3.6 Comparisons of platelet indices among type 2 diabetic patients treated with metformin and Glibenclamide and with healthy controls by Kruskal Wallis test and Post hoc test

With the Kruskal-Wallis test, the median (IQR) values of PLT count, PCT, MPV, PDW, and PLCR were significantly different between the type 2 diabetic patients treated with Metformin, in type 2 diabetic patients treated with Glibenclamide and healthy controls ($p<0.05$)(**Table** 6).

As shown by the Bonferroni test, PLT and PCT values showed no significant difference between metformin-treated T2DM and healthy controls ($p=1.0$ for PLT count and $p=0.31$ for PCT), respectively. Whereas PDW, MPV, and PLCR were significantly increased in type 2 diabetes treated with metformin compared with controls ($p<0.001$). No significant differences were observed between type 2 diabetic patients treated with glibenclamide and metformin in PDW and PCT values. While, MPV at ($p=0.023$) and PLCR at ($p=0.045$) were elevated in type 2 diabetic treated with metformin and PLT count at ($p=0.006$) was increased in type 2 diabetic patients treated with glibenclamide **(Table** 6**).**

All platelet indices were significantly elevated in glibenclamide-treated type 2 diabetic patients compared with healthy controls ($p<0.05$). There is elevated level of platelet indices in type 2 diabetic patients on oral hypoglycemia therapy compared with healthy control group. Since our study is cross-sectional design we couldn't assess before treatment of PLT indices status, dosage and duration of treatment. Also patient adherence to the drugs was not assessed and others factor may affected the drug's effectiveness and thereby cause for elevations of PLT indices in patients **(Table** 6**).**

Table **6:** Kruskal Wallis and Bonferroni pairwise comparisons of platelet indices across DM patients treated with oral hypoglycemia drug and healthy controls at Bishoftu Hospital, from June to August 2022.

Platelet indices	Glibenclamide treatedT2DM Median(IQR)	Metformin treatedT2DM Median(IQR)	Healthy controls median(IQR)	Kruskal Wallis test	Post hoc test (Bonferroni test)		
				p-value	P*-value	P**-value	P***-value
PLT count (10^3/lμ)	235(179-313)	188(157-231)	208(170-132)	0.007	1.000	0.006	0.037
PDW(Fl)	13(11.7-16.3)	14.8(12.3-22.6)	11.1(10.1-13.1)	P<0.001	P<0.001	0.295	P<0.001
MPV(Fl)	10.7(10.1-12.1)	11.6(10.6-14)	9.9(9.6-10.9)	P<0.001	P<0.001	0.023	0.008
PLCR%	30.4(25.7-40.4)	37.1(29.5-52.1)	24.5(21.2-31.8)	P<0.001	P<0.001	0.045	0.003
PCT%	0.25(0.21-0.35)	0.23(0.2-0.28)	0.23(0.19-0.26)	P<0.001	0.31	0.216	0.006

Key Note: The p-value was significant at p-value<0.05, while:-

***P**-stands for comparison of T2DM treated by metformin and healthy control group.

****P**-stands for comparison of T2DM with Glibenclamide and Metformin.

*****P**-stands for comparison of healthy control and T2DM treated with glibenclamide

5.3.7 Correlational analysis of the platelets indices with BMI, WC, and WHR among study participants.

In correlation analysis, PDW (p=0.033), PLCR (p=0.018), and PCT(p=0.009)values were positively correlated with BMI in type 2 diabetic patients. On the other hand, in T2DM, the PLT count (p=0.011) was positively correlated with WHR, and the PCT value was also significantly positively correlated with WC (p=0.006) and WHR (p<0.001), in T2DM.Anthropometric measurements did not show any statistical significant correlation with the platelet indices in the control group (**Table** 7)

Table **7:** Spearman's correlation (rho) of platelet indices with BMI, WC, &WHR among T2DM patients and healthy controls at Bishoftu Hospital from June to August 2022.

PLT indices	T2DM patients group			Healthy control group		
	BMI rho(p)	WC rho(P)	WHR rho(p)	BMI rho(P)	WC rho(P)	WHR rho(P)
PLT	0.092(0.227)	0.117(0.124)	0.192(0.011)*	-0.209 (0.052)	0.079(0.467)	0.075(0.49)
PDW	0.162(0.033)*	0.063(0.408)	0.072(0.348)	0.126 (0.246)	-0.117(0.282)	-0.129(0.234)
MPV	0.132(0.081)	0.058(0.445)	0.033(0.667)	0.142 (0.188)	-0.043(0.692)	-0.049 (0.652)
PLCR	0.179(0.018)*	0.044(0.568)	0.016 (0.833)	0.135 (0.212)	-0.082 (0.448)	-0.076 (0.483)
PCT	0.199(0.009)**	0.208(0.006)**	0.298(0.001)**	-0.088(0.417)	0.083 (0.444)	0.159 (0.142)

Key Notes: **Correlation is significant at 0.01 level; *correlation is significant at 0.05 level (two-tailed); rho is the spearman's correlation coefficient.

5.3.8 Correlational analysis of the platelets indices with clinical variables and Blood pressure among study participants.

Regarding clinical variables, PDW, MPV, and PLCR values achieved positive and significant correlations with SBP at (p=<0.001). On the other, PDW, MPV, PLCR and PCT showed a significant positive correlation with DBP. Our data also showed that PDW, MPV, PLCR, and PCT were significantly positively correlated with FBS; whereas, PDW, MPV, and PLCR showed a significant positive correlation with DM illness duration at (p<0.001), in type 2 diabetic patients. On the other hand, no significant correlations between all platelet indices and clinical variables were found in the healthy control group (p>0.05) (**Table** 8).

Table **8:** Spearman's correlation (rho) of platelet indices with SBP, DBP, FBS &duration of DM illness among T2DM and controls group at Bishoftu Hospital, from June to August 2022**.**

Platelet indices	Diabetic patients group				Healthy control group	
	SBP rho(p)	DBP rho(P)	FBS rho(p)	Duration rho(P)	SBP rho(P)	DBP rho(P)
Platelet count	07(0.358)	0.067(0.378)	0.048(0.529)	0.133(0.137)	-0.024(0.826)	-0.071(0.511)
PDW	0.416(0.001)**	0.44(0.001)**	0.525(0.001)**	0.351(0.001)**	-0.12(0.27)	0.031(0.779)
MPV	0.355(0.001)**	0.398(0.001)**	0.474(0.001)**	0.298(0.001)**	-0.061(0.575)	-0.005(0.964)
PLCR	0.421(0.001)**	0.394(0.001)*	0.509(0.001)**	0.372(0.001)**	-0.059(0.586)	-0.005(0.965)
PCT	0.12(0.113)	0.206(0.006)**	0.164(0.03)*	0.078(0.304)	-0.036(0.738)	0.139 (0.198)

Key Notes: **Correlation is significant at 0.01 level; *correlation is significant at 0.05 level (two-tailed); rho is the spearman's correlation coefficient.

5.3.9 Determination of predictive values of platelet indices as indicators for poor glucoregulation in a diabetic patient by ROC analysis.

An analysis was performed to determine the ability of the platelet indices as predictors of poor glycemic control in diabetic patients. Thus, at a cut-off value ≥15.75fl with a sensitivity of 68%, a specificity of 90%, a PPV of 87.2%, and an NPV of 73.8% with an AUC of 0.803($p<0.001$), the PDW can differentiate poor glycemic-controlled type 2 diabetic patients from good glycemic controlled type 2 diabetic patients. Also, MPV can distinguish poor glycemic-controlled and good controlled type 2 diabetic patients with a sensitivity of 61%, a specificity of 93%, PPV of 89.7%, and NPV of 70.5% with an AUC of 0.774, with a cut-off value ≥12.25fl at($p=0.001$).

The PLCR can distinguish poor glycemic control from good controlled type 2 diabetic patients at a cut-off value ≥36.3% with a sensitivity of 69%, a specificity of 83%, a PPV of 80.2% and an NPV 72.3% with an AUC of 0.775 at ($p<0.001$). Also, the ROC analysis demonstrated that PCT can differentiate poor glycemic-controlled from good controlled type 2 diabetic patients at a cut-off value of ≥0.24 with a sensitivity of 83%, a specificity of 57%, a PPV of 65.9% and an NPV of 87.7% with an AUC of 0.761 at ($p<0.001$).In our data from ROC analysis, the PLT count couldn't distinguish between poor glycemic control and good control in patients with type 2 diabetes (**Table** 9 and **Figure** 3).

Table **9:** Determination of predictive values of platelet indices for poor glycemic control of T2DM patients attending at Bishoftu Hospital, from June to August 2022.

Platelet indices	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Cut-off value	AUC	95%CI	P-value
PDW(fl)	68%	90%	87.2%	73.8%	≥15.75	0.803	(0.735,0.871)	<0.001
MPV(fl)	61%	93%	89.7%	70.5%	≥12.25	0.774	(0.702,0.847)	<0.001
P-LCR(%)	69%	83%	80.2%	72.3%	≥36.3	0.775	(0.704,0.838)	<0.001
PCT (%)	83%	57%	65.9%	87.7%	≥0.24	0.761	(0.69,0.831)	<0.001

Key Note: PPV-positive predictive value, NPV-negative predictive value, AUC-area under a curve, 95%Cl-at 95% confidence interval.

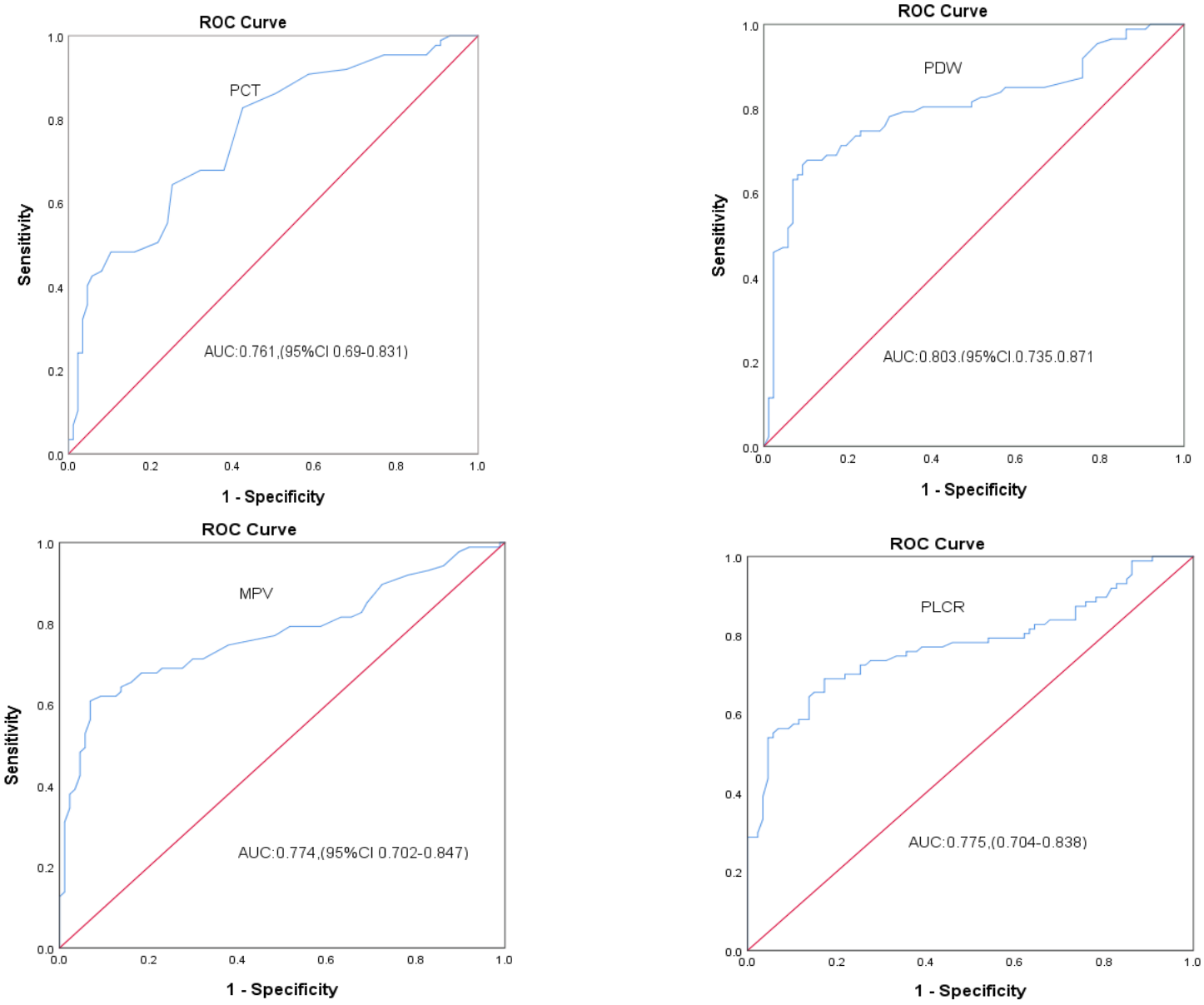

Figure **3**: The ROC curve analysis of PLT indices as a predictor of poor glycemic control in type 2 diabetic patients attending Bishoftu General Hospital, from June 15 to August 12, 2022.

CHAPTER SIX: DISCUSSION

Failure to control diabetes in its early stages leads to vascular complications due to metabolic & blood component disturbance(3). Insulin resistance has been found to cause hyperglycemia which results in platelet activation(1,6,7).The Platelet indices have got great attention in diabetic patients because of they are used as an alarm to diagnose,initiate, or progress diabetic vascular complications in a simple, rapid and inexpensive manner(16).

This study result indicates that there was no significant difference in PLT count between diabetic and control groups. This is in line with studies conducted in Ethiopia(42),China(44),Iraq(46),Sudan(47),and Egypt(36).In contrast to this study, studies in Ethiopia(43)and Nigeria(48), found that PLT count in type 2 diabetes was significantly higher than in controls. Possible reason for the discrepancy could be due to difference in sample size in which those studies contradicts with our study result used small sample size than our study.

Furthermore, there was no significant difference in PLT counts between poor glycemic control type 2 diabetes, good glycemic control diabetes and healthy controls. Similarly, reports from a study conducted in India(38) found no significant differences between groups.Although there is a tendency of platelet production in type 2 diabetes, a possible explanation for the comparable results in platelet count between these groups might be because of rapid consumption of activated platelets rather than the production since there are study participants with the events of microvascular complications(35).

Results of the current study revealed elevated PCT levels in diabetic patients compared to control. Plateletcrit is a parameter calculated from PLT count and MPV, it is also considered as a guide to prevent vascular complications due to glycemic dysregulation. Platelets play a central role in the inflammatory process.However,plateletcrit plays a better role than platelet count in assessing the inflammatory status in diabetic patients,and its high value is indicative of inflammatory status in diabetic patient(18,24).Our findings were consistent with studies conducted in India(37) and Brazil(34).However, it was contrasted with a study conducted in Egypt (36).The discrepancy might be due to difference in sample size in which study done in Egypt used small sample size than our study.

Moreover, the current study found higher PCT value in diabetic patients with poor glycemic control than with good glycemic control diabetes and healthy control. Similar results were found in studies conducted in India(35,37).On the other hand, a study conducted in India(38) found contradictory results to our findings. This discrepancy may be due to difference in sample size and study period. Other reasons could be platelet aggregation due to poor venipuncture, tube overfilling, and poor mixing of the sample(14).

According to this study findings, PCT was identified as a discriminator between poor glycemic control type 2 diabetes and good glycemic controlled type 2 diabetes. Based on its AUC value, it's a good predictor of poor glucoregulation in type 2 diabetic patients (17). To best of our literature browsing we couldn't found the study done to determine the predictive ability of PCT for poor glucoregulation that constrained as to compare and contrast our findings.

In our study, PDW was increased in type 2 diabetics than in healthy controls. The possible increment of PDW may be due to, in vivo protein kinase C activation and non-enzymatic glycation of platelet surface proteins resulting in decreased platelet membrane fluidity and increase activation(7).The activated platelets in T2DM patients differ in size from the inactive ones and there is the formation of pseudopodia structures that lead to shape changes from biconcave discs to spheres and bring changes in the PDW. This increases the base of the histogram plot of platelet distribution to be increased(34).

Studies conducted in Egypt (36),Turkey (41), India(31), and Brazil (34),reported the onsistent result with our study findings.On the other hand,the results of studies conducted in China(44) and Ethiopia(43), contradicted with our study results. The generated difference might be due to the variation in sample size and study design between this study and studies contradicted with our findings.

Moreover,PDW was significantly elevated in diabetic patients with poor glycemic control than in diabetic patients with good glycemic control and healthy control.The consistent report with our findings were raised from studies conducted in India(31,35,37,38),Greece(40), Brazil(34),and Egypt(36).

In our study, the results ROC curve analysis showed that PDW could distinguish the poor glycemic control status from good controlled in T2DM.As a result, indicates it is an excellent predictor of poor glycemic control in diabetic patients. This study results showed,a slightly higher cut-off value, lower sensitivity, PPV, and specificity values of PDW compared to a

study done in India(31).A study conducted in Bangladesh (32) reported lower values for all parameter of the ROC curve output as equated to ours. Possible reason for this contradiction could be due to inadequate glycemic control in our study despite the use of treatment and difference in sample size and study design.

The current study found that diabetic patients had increased MPV values than nondiabetic co ntrols. The background reasons for platelet activation in DM is, the chronic hyperglycemia ca n lead to nonenzymatic glycation of platelet surface proteins. Elevated blood glucose and some glucose metabolites also cause platelet osmotic swelling, increasing platelet reactivity a nd shortening platelet lifespan, reflecting the recruitment of young, large platelets(38).Report s from the studies conducted in the Egypt(36) and Brazil(34) came up with consistent results with our study. Contrary to our study results,studies conducted in Ethiopia(43), Iraq(4 6),and Sudan (47), reached different conclusions. The possible reason for the discrepancy might be due to the different in sample size, study design and study period.

Also, this study showed that MPV was significantly increased in diabetic patients with poor glycemic when compared with diabetic patients with good glycemic control and non-diabetic controls.This finding was consistent with studies conducted in India(35,37),Egypt (36),Brazil (34), and Greece(40).However, a report from a study in Turkey(50),contradicts our report. The disagreement occurred between ours and study from Turkey may be due to variation in study designs. Our study used the primary data. However, the study done in Turkey used secondary data.

Current study found that MPV to be as a good predictor of poor glycemic control in type 2 diabetes. According to ours MPV showed elevated cut-off,specificity, and PPV values compa red with a study conducted in India(31), and a study conducted in Bangladesh came up with lower all ROC curve output than ours(32).On the other hand, a study conducted in Bosnia and Herzegovina (33), showed a lower cut-off value ,specificity, higher sensitivity values, and AUC value comparable with ours. This inconsistency may be due to difference in study period and differences in sample size.

The results of this study show that P-LCR was significantly higher in diabetic patients compared with controls. The possible explanation of PLCR in cases than control group is, when platelet activated in DM there is release of largest platelet fraction which are usually measured as PLCR. The PLCR indirectly correlates with PLT counts, and directly with MPV and PDW, but is more sensitive to increased platelet size(24).

Our finding coherent with studies in Brazil(34),India(35), and Egypt (36).In contrast to our re sults,a study conducted in Ethiopia (43) reached different conclusions. The disagreement may be due to the newly diagnosed diabetic patients involved in the previous studies. In newly diagnosed diabetes, inflammation and hyperglycemia are almost at near normal levels, which do not lead to changes in platelet activation or increase of platelet indices.

Also, significantly higher PLCR was observed in diabetic patients with poor glycemic compared with good glycemic control diabetics and healthy controls. Similar reports emerged from studies conducted in India(35,38).

In our study, PLCR was identified as a predictor of poor glycemic control in diabetic patients. As a result, following the general rule of thumb for interpreting AUC to evaluating the diagnostic ability of a test in discriminating the real aliment status of a patient, PLCR was showed as a good parameters for prediction of poor glycemic based on its AUC value(17). The PLCR is not often quoted in the literature, probably because it is relatively a newly invented PLT indices. Mostly it is generated by a few machines, with Sysmex analyzer generations(39).

In our study, PDW, MPV and PLCR were significantly elevated in complicated T2DM with compared to uncomplicated T2DM. Our study results were in line with studies conducted in Brazil(34),India(35),and Belgaum(39). Diabetes mellitus is a multi-systemic disease that affects the eyes, kidneys, and peripheral nerves and leads to micro- and macroangiopathy through chronic hyperglycemia that enhance the platelet indices alteration in diabetic patients with complication(24).A study from Pakistan(49) reached conflicting conclusion with our findings. A possible explanation for the difference could be due to the differences in onset of complications in the current and previous study populations. Since complications can exaggerate platelet activation over time. Also the difference may be due to sample size difference in which our study uses larger sample size than study done in Pakistan.

Regarding the correlation between platelet indices with anthropometric measurements, the results of current study showed significant positive correlations between PDW, PLCR, and PCT with BMI, and PLT count with WHR, and PCT with WC and WHR showed a positive si gnificant correlation. Possible explanation for the correlation between platelet indices and anth

ropometric is,studies have shown that BMI, WC, and WHR are indicators of diabetes disorde rs like obesity.Obesity is abnormal accumulation of adipose tissue caused due to a metabolic disorders such as dyslipidemia, hypertriglyceridemia, and hypertension.This disorders result in various cytokines changes that promote platelets activation and PLT indices alterations(9,1 0).

Contrary to this study findings, a study conducted in Ethiopia (42) found that PDW,MPV and platelet count were negativey correlated with BMI and WHR($p>0.05$).This inconsistency ma y be due to differences in procedures measurements of BMI,WC, and WHR between study populations and difference in study period.

As spearman correlation with respect to blood pressure in our findings, the PDW, MPV, and PLCR were significantly positively correlated with SBP. While, PDW, MPV, PLCR, and PCT were showed a significant positive correlation with DBP.The possible reasons for correlation is the hyperglycemia in DM causes the larger platelets to be released into circulation.Increase d formation of reticulated platelets leads to complications of atherothrombosis in diabetic pati ents(25,26).All of the above points lead to increased vasoconstriction variability and changes in blood vessel properties, ultimately leading to platelet activation and changes in PLT indice s. Consistent with our finding, a study conducted in Turkey (23) also found that PDW was significantly and positively correlated with DBP, and a study conducted in Ethiopia (42) foun d that PLT count and MPV were positively correlated with SBP.

The results of our study showed a significant positive correlation between PDW, MPV, PLCR, PCT and fasting blood glucose levels. This finding supports evidence for a strong relationship between glycemic status and platelet hyper activation and suggests a possible etiology of vascular complications in diabetes(3,6,7).The results of our study are concordant with a study conducted in India that showed that FBG levels were positively correlated with PDW, MPV,and PLCR, whereas PLT count and PCT doesn't correlated with FBG(38).Another study from India(31) found that PDW was positively correlated with FBG. A study conducted in Brazil(34), also showed positive correlations between MPV, PDW, PCT and FBG, and a study from Ethiopia(42) showed positive correlation between MPV and FBG. The negative correlation obtained in the study done in India(38)may be due to significantly decreased values of the PLT count that cannot compensate the MPV increment result in lower PCT.

In the current study, PDW, MPV, and PLCR were significantly positively correlated with DM disease duration. A possible explanation for the correlation between the platelet indices and

duration of diabetes illness is that vascular complications increase with longer disease duration, leading to endothelial dysfunction and subsequently increased platelet activation in adult patients with type 2 diabetes(51). Following with our findings, a study conducted in India(35) found that PDW and MPV were significantly and positively correlated with duration of DM disease, and a study conducted in Ethiopia(42) found that PLT and MPV were significantly and positively associated with duration of DM disease. Another study from Ethiopia(43) report PLT correlation with diabetic illness duration.

Strengths and Limitations of our study

Strength of this study

- This study is a comprehensive study to evaluate nearly all platelet indices in various conditions of type 2 diabetic patients and it could give clue on the purpose of platelet indices in predictions of poor glucoregulation in diabetic patients.

- Morphological analysis for the platelet picture characterization was also done to check the automation results.

Limitations of this study

- This study used a cross-sectional study design that limits the ability to identify causal relationships between the platelet indices and factors these significantly correlated with platelet indices.
- Assessment of coagulation profile and immature platelet fraction was not done, and plasma glucose was used instead of HgbA1c due to limitations of HgbA1c test in the Hospital.

CHAPTER SEVEN: CONCLUSION AND RECOMMENDATIONS

7.1 Conclusion

In conclusion, our study showed that PDW, MPV, PLCR, and PCT were significantly increased in diabetic patients compared with controls. Moreover, the PDW, MPV, PLCR, and PCT values were significantly elevated in poor glycemic-controlled T2DM patients than in good-controlled T2DM patients and healthy controls. Also, PDW, MPV, and PLCR were significantly elevated in the complicated T2DM patients compared with uncomplicated T2DM. At the different levels the PLT, PDW, MPV, PLCR, and PCT were significantly correlated with anthropometric measurement. In addition, the PDW, MPV, PLCR, and PCT were strongly and significantly correlated with fasting blood glucose, blood pressure, and duration of DM illness. Also, PDW, MPV, PLCR, and PCT have also been identified as potential predictors of poor glucoregulation in diabetic patients.

7.2 Recommendations

The following recommendations were given based on the above results to the concerned bodies:

For physicians and DM patients: Give close attention to platelet indices abnormalities during follow-up and monitoring of DM is strongly recommended to mitigate diabetes related disorders, and is better to use PLT indices in combination to diagnose DM that could offer a more lawful diagnosis value since they reimburse for each other's confines. It is also essential for diabetics to follow their physician's advice, guidance, and direction to check their platelet indices profile to prevent or slow down diabetes vascular complications.

For policymakers and administrators: It is encouraged in the area to take part in the advancement of DM management protocols and strategies that cogitate the platelet indices as prognostic tools during DM follow-up and monitoring programs, especially in the limitation HgbA1c test setup. Also, it's very important to allocate resources such as free CBC services for DM Patients to facilitate the follow-up and monitoring procedures simply and cost-effectively.

For researchers: our recommendation for researcher is to conduct large scale longitudinal study to evaluate whether PLT indices are predictor of DM related disorders. In addition to PLT indices, determination of immature platelet fraction and coagulation profiles also required to convince views of PLT activation in diabetes. The relationship between blood glucose and PLT indices should be assessed by using HgbA1c than fasting blood glucose.

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ANNEX

Annex I: Information Sheet

I. English version

Title of the Research: Assessment of platelet indices and its predictive value in type 2 diabetes mellitus adult patients attending at Bishoftu General Hospital, central Ethiopia, 2022.

Principal Investigator name: Dereje Abebe

Advisors: Mr. Girum Tesfaye(BSc, MSc, Assistant professor, Ph.D. candidate)

Mr.Gebeyaw Arega(BSc, MSc)

Organization: Jimma University (School of Medical Laboratory Science)

Sponsor: Wolkite University

Introduction: This information sheet holds all information about the research titled above. It gives you needed information about the whole processes that will be undertaken in the study and your participation is based on your decision.

Description and Purpose of the study

Diabetes is a chronic metabolic disorder of multiple etiologic factors characterized by the presence of chronic hyperglycemia with disturbance of carbohydrate, fat, and protein metabolisms.

It is the world's major public health challenge, affecting individuals, and families, and threate n the well-being of entire communities. Hyperglycemia is a common effect of uncontrolled type 2 diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels.

Determining the levels of platelet indices in type 2 diabetes mellitus is important to make an information-based decision and to make an intervention. To the best of the principal investigator's knowledge, there is limited platelet indices in type 2 diabetes mellitus adult patient's evidence in Ethiopia, particularly in this study area. Hence, this study was designed to assess platelets indices in type 2 diabetes mellitus adult patients attending at Bishoftu General Hospital, central Ethiopia, 2022.

Procedures

In conducting the above study, we would like to inform you that we have some questions regarding sociodemographic and others physical measurements and samples for laboratory

testing. If you agree to participate, provide samples, and provide information required for the study; we take guarantee that the collected samples is processed in the central laboratory of Bishoftu General Hospital.

Discomforts and Risks

Mild pain and discomfort may occur during a venous blood draw. But surely you relieve within minutes and a blood sample will be collected according to standard operational procedures (SOPS).

Benefits and Compensation

There are no direct financial benefits from this study. However, based on your test results, you may be referred to your doctor for further care and treatment**.**

Confidentiality

Regarding to the collected information confidentiality, surly I inform you that the records will be kept in locked cabinets, and the results of the tests will be coded for identification purposes than using your names and collected samples not used for other purposes & safely disposed of after the completion of the study.

Right to refuse or withdraw and whom to contact

Lastly, I inform you that you have full right to withdraw from the study at any time and you are not be discriminated against in any form of health services due to your refusal. By using the following contact addresses you can contact investigators at any time.

Mr. Dereje Abebe, Phone +251-903036210, Email: sifaanabebe@gmail.com

Mr. Girum Tesfaye, Phone +251-920-274035, Email:girumtesfaye12@gmail.com

Mr.Gebeyaw, Arega, Phone +251-931858197,Email:gebearega@gmail.com

Annex II: Consent form

I. English Version

Participants name _____

I have informed in the language I understand about the aim of the above mentioned research. I understood the purpose of the study titled with assessment of platelet indices in type 2 diabetes mellitus adult patients attending at Bishoftu general hospital, central Ethiopia, 2022 GC. I informed about specimens that could be taken and the existence of interviews. In addition, I have been told all the information collected throughout the study process is kept in confidential. I understood that my current and future medical services will not be affected, if I refused to participate or withdraw from the study.

Agree_____Not agree_____

Therefore, I give my consent freely for my participation in this study.

Name of participant: _____Signature:_____ Date_____

Name of researcher: _____Signature_____ Date_____

Annex III: Questionnaires

I. English version

- **For T2DM group and General instructions**

Participants Card no: _____

Participants Code: _____

- Please encircle your responses from a given alternative

S.no	**Questions**	**Possible answers**
	Part I. Socio-demographic variables	
1.1	Age in full years?	_____
1.2	Sex	0. Male 1.Female
1.3	Where is your residence?	0. Urban 1. Rural
1.4	What is your education level?	0. Unable to read and write 1.Can read and write 2.primary school 3. secondary school 4.higher education
1.5	What is your occupation?	0. Unemployed 1. merchant 2. farmer 3. government employee
1.6	What is your marital status?	0. Single 1. Married 2. Divorced 3. Widowed
1.7	What is your monthly income in (ETB)?	0. <1500ETB 1. 1500-6000ETB 2. >6000ETB

Part II. Anthropometric and BP measurements		
2.1	Weight of participants in Kilogram	_____Kg
2.2	Height of participants in meters	_____m
2.3	Body mass index of participants in Kg/m^2	_____Kg/m^2
2.4	Hip Circumference of participants in cm	_____cm
2.5	Waist Circumference of participants in cm	_____cm
2.6	Waist to Hip Ratio of participants	_____
2.7	Blood pressure (BP) of participants in mmHg	1.Systolic_____mmHg 2.Diastolic_____ mmHg
Clinical and behavioral characteristics(for exclusion or inclusion participants)		
1	Have you history of malarial infection in last two months?	0.no 1.yes
2	Have you habit of smoking cigarette?	0.No 1.yes
3	Have you alcohol drinking habit?	0.no 1.yes
4	If yes for Q3, how much do you drink per day?	0.greater than two unit drinks(exclude from study) 1.less than two unit drinks(include in study)
Name of data collector_____Date_____sign_____		

- **Checklists**

These checklists prepared to collect clinical variables from the medical record of type 2 diabetic patients attending at Bishoftu General Hospital, central of Ethiopia.

S.no	Clinical variables	Possible options
1.1	Current Oral hypoglycemic therapy used	1. 2. 3.
1.2	Duration of DM since diagnosed	0. <7years and mention in years_____ 1. >7 years and mention in years_____
1.3	FBS levels in last two months	month1_____mg/dl month2_____mg/dl
1.4	Presence of microvascular complication at least one type.	0.no 1.yes
Name of data collector_____Sign_____Date_____		

Questionnaires for the control group and general direction

- Participants Code: _____
- Please encircle your responses from a given alternatives.

S.No	Questions	Possible answers
	Part I. Socio-demographic variables	
1.1	Age in years	_____
1.2	sex	0.Male 1.Female
1.3	Where is your residence?	0. Urban 1. Rural
1.4	What is your educational level?	0. Unable to read and write 1.Can read and write 2.primary school 3.secondary school 4.higher education
1.5	What is your occupation?	0. Unemployed 1. merchant 2. farmer 3. government employee
1.6	What is your marital status?	0. single 1. married 2. divorced 3. widowed
1.7	What your monthly income in (ETB)?	0. <1500ETB 1. 1500-6000ETB 2. >6000ETB
	Part II. Anthropometric and BP measurements	**Measured values**

2.1	Weight of the participants in kilogram	_____Kg
2.2	Height of participants in meter	_____m
2.3	Body mass index of participants in Kg/m^2	_____Kg/m^2
2.4	Hip Circumference (HC) of participants in cm	_____cm
2.5	Waist Circumference (WC) of participants in cm	_____cm
2.6	Waist to hip ratio of participants	_____
2.7	Blood pressure (BP) of participants in mmHg	1.Systolic_____mmHg 2.Diastolic_____ mmHg
Clinical and behavioral characteristics(to exclude or include study participants)		
1	Have you history of malarial infection in last two months?	0.no 1.yes
2	Have you habit of smoking cigarette?	0.no 1.yes
3	Have you alcohol drinking habit?	0.no 1.yes
4	If yes for Q3, how much do you drink per a day?	0. greater than two unit drinks(exclude from study) 1. less than two unit drinks(include in study)
Name of data collector_____Date_____Sign_____		

Dabalata I: Waraqaa Odeeffannoo

Mata-duree Qorannicha: Hospitaala waligala Bishooftuutti waldhanamtoota dhukkuba sukkaara gosa lammaffa qaban keessattii bu'aa qorannoo laaboratoorii gosa dhiigaa kantarsaa (platileetii).

Maqaa qoraata: barataa Darajjee Abbabaa

Gorsiitoota: Barsiisaa Gurum Tasfayyee(Masteerii garg.piroffesara,kadhimamaa Ph.D.)

:Barsiisaa Gabayyoo Araggaa(BSc,MSc)

Maqaa dhabbaaticha: Yuuniversiiti Jimma, Instituyuuti Fayyaa,Faakaaltii Saayiinsi Fayyaatti,Kutaa Meedikaala Laabooratoorii.

Qaama Ispoonsara godhee: Walqixxee Yuuniversiiti.

Seensa: Waraaqan oddeeffaanno Kun waa'ee qorannoo mata-dureen isaa olitti xuqamee hundumaa kan qabateedha.Oddeeffannoo barbaachisaa akkataa adeemsaa qorannichaa haalan kan ibsuu fi hirmaannan keessaan immoo fedhii irratti kan hundaa'ee tahuu isiniif ibsuun barbaada.

Ibsa fi barbaachisumma qorannichaa

Dhibeen sukkaara dhukkubaa daddaarba kan hin taanee fi sababoota garagaaran dhiigaa keenyaa keessattii hammii suukaara garmalee akka baayyatuu kan godhuudha.Yeroo amma kanattii dhibeen kun babal'achaa kan jiru fi rakkoo fayyaa nama dhuunfaa, maatii fi hawwasaa bal'aa tahee addunyaa kana raasa jira.Dhiigaa keessaatti garmalee bayyachuun sukkaara sirnoota qaama kan miidhuu yoo tahuu,keessuma immoo narvii fi ujjuummolleen dhiigaa caalattii kan miidhuudha.

Hamma gosa dhiiga pilatileti(platelet indices)warrotaa dhibee sukkaara gosa lammaffa qabani beekuun, osoo dhibichii qaama keessaatti babaal'atee balaa hammaaf nama hin kennin to'aachuuf baayyee gargaara.

Hmman oddeeffaannoo argaadheettii qoraannoon armaan duraa mata-duree kanarrattii xiyyeeffatee Itiyoophiyya,keessuuma immoo Bishooftuu keessaattii gaggeeffaamee murasaa yoo tahuu, sababaa kanaf qorannoon kun qophaa'ee Hospitaala Bishooftuuti kan gaggeeffamuu fi hirmaanan keessaan immoo murteessa waan ta'eef fedhii keessaanin akka irrattii hirmaatan.

Haala adeemsaa qo'annicha

Qorannoo armaan olitti eeramee kana galmaan gahuuf gaaffileen waa'ee haawwasumma ilaalan fi kan biroo, qaama safaruuni, fi samuudnii laaboratooriif barbaarchisuu akka jiru isiinif ibsuun barbaada.Saamudnii isin irraa fuudhamuu immoo Hospitaala waligaala Bishooftuu kutaa labooratoorii keessaattii qoraatama.

Sodaa fi miidhaa qorannoo

Dhukkubbiin salphaan fi miira gaarii dhabuun yeroo dhiiga ujummoo dhiigaa fudhatamu uumamuu danda'a. Garuu daqiiqaa muraasa keessatti boqonnaa akka argattu beekamaadha, akkaataa adeemsa hojii istaandaardii (SOPS) tiin saamuda dhiigaa ni sassaabama.

Faayidaa qu'annichaa fi kaaffaltii hirmaataaf godhamu

Qorannoon kun faayidaan maallaqaa kallattiin hin jiru. Haa ta'u malee, bu'aa qorannoo keessanii irratti hundaa'uun kunuunsaa fi yaala dabalataaf gara hakiima keessaniitti ergamuu dandeessu.

Iccitii qo'annichaa

Iccitin firii qorannoo samuudaa hundii isaani haala gaariin kan eegaman tahu.Akkasumas rag ootni fudhataman hundinu nama raga sassabeen maqaa keessaniin osoo hin taane lakkoofsaa koodii adda ta'een kan galmaa'uudha.

Mirga fedhaan hirmaachu fi teessoo oddeffannoo

Qu'annoo kana irratti hirmaachun fedha keessaan qofa irrattii kan hunda'ee ta'uusaa beektaa ni, yeroo barbbaaddanitti dhiisu akka dandeessaan mirgii keessaan eeggamadha. Qu'annoo ka na irratti hirmaachu fi hirmaachu dhiisuu keessanin ammas tahee gara fuuladuraaf tajaajila w aldhaansa argattan kammiiyyuu irratti rakkoo tokkollee isinitti hin fidu.Qu'annicha ilaalchise e gaaffilee qabdan hundaa yeroo barbaaddanitti teessoo gaditti barreeffameen abbaa qu'annic haa gaafachuu dandeessu.dhumarratti qu'anno kanaaf hirmaannaa waan gootanif galanni kee nyaa guddaadha.

Baraata: Darajjee Abbabaa, Phone +251-903036210, Email: sifaanabebe@gmail.com

Barsiisa: GuruumTasfaayyee,Phone+251-920-274035,Email:girumtesfaye12@gmail.com

Barsiisa: Gabayyoo Araaga, Phone +251-931858197,Email:gebearega@gmail.com

Dabalata II: Unka hayyamaa (hiika Afaan Oromoo)

Lakk. addaa hirmaata_____

Maqaa guutuu hirmaata_____

Ani hirmaataan maqaan kiyya armaan olitti ibsame kaayyoon qo'annoo mata-dureen isa: hospiitaala waligala Bishooftuutti bu'aa qorannoo laaboratoorii gosa dhiiga kantarsa (pilaatile ti) warreen dhukkuba sukkaara gosa lammaffaa qabani fi waldhaansarraa jirani beekuu, jedhu uratti hirmaachuuf afaan naa galuun odeefannoo gahaa argadheen jira.Odeefannoon fayyaa fi naamuusni samuudni ittin fudhataamuu karaa miidhaa/rakkoo hin geesisneen akka tahee huba dheen jira. Kana malees, odeeffannoon adeemsa qo'annoo guutuu keessatti walitti qabame hundi iccitiidhaan akka eegamu natti himameera.Gaaffilee gaafatamuuf deebii kennu dhiisuu, hirmaachuu dhiis uu fi yeroon barbaaddetti addaan kutuu akkan danda'uu hubadheen jira.Kana gochuu kiyyaan is ammas ta'e gara fuuladuraaf fayyadamummaa tajaajila fayyaa kiyyaa irratti rakkoon tokkol lee akka hin uumamnee naaf galee jira.

Walii galeera_____walii hin gallee_____

Kanaafuu, qoorannoo kana iratti feedhii koon nan hirmaadha.

Maqaa hirmaataa_____Mallattoo_____Guyyaa_____

Maqaa qoo'ataa_____Mallattoo_____Guyyaa_____

Dabalata III: Gaaffiilee (hiikaa afaan oromoo)

- **Gaaffilee garee wal'anamtoota dhibee sukkaara gosa lammaffaf qophaa'ee**

Lakk.kaardii hirmaata: _____Koodii adda hirmaata: _____

Kallaattii waliigalaa:Maaloo gaaffilee filaannoo qabaniif,filaannoo keessaanin nuuf mirkane essa.

Lakk.	Gaaffiillee	Filaannoo
	Kutaa-I:Gaaffiwwaan haasummaa waliin walqabatan	
1.1	waggaa guutuun Umriin kee meeqa?	_____
1.2	Saala/koorniyaa	0. Dhiiraa 1.Dhalaa
1.3	Bakki jireenyaa keessan eessa?	0. Magaala 1. Baadiyyaa
1.4	Sadarkaan barnootaa keessaan maal fakkaata?	0.dubbisuu fi barreessuu hin dand'uu 1.dubbisuu fi barreessuu nan danda'a 2.sadarkaa tokkoffaa 3. sadarkaa lammaffaa 4.sadaarka olaanoo
1.5	Hojiin keessan maali?	0.Hojjii hin qabu 1.daldalaa 2.qotee bulaa 3. Hojjataa/ttu mootummaa
1.6	Haallii gaa'elaa keessanii maal fakkaata?	0. Kan hin heerumnee/fuune 1. Kan heerumtee/fuudhee 2. Kan addaan baahan 3. Kan abban warra irra darbee(du'e)ykn haati warra jalaa darbitee(duute)

1.7	Galiin ji'aa keessan (ETB)?	0. <1500 Qr.Itiyoophiyya. 1. 1500-6000 Qr.Itiyoophiyya. 2. >6000 Qr.Itiyoophiyya.
	Kutaa-II: Safartoo Anthropometric fi Dhiibbaa Dhiigaa	**Bu'aa safara qaama**
2.1	Ulfaatina hirmaattotaa Kiiloogiraamiin	_____Kg
2.2	Hojjaa qaama hirmaattota	_____m
2.3	Indeeksii ulfaatina qaamaa hirmaattootaa Kg/m2	_____Kg/m^2
2.4	Naannawa Luqqeettuu	_____cm
2.5	Naannawwa Mudhii	_____cm
2.6	Reeshiyoo naannawa mudhii fi luqqeettuu	_____
2.7	Dhiibbaa dhiigaa hirmaattoota mmHg	1."Systolic"_____mmHg 2."Diastolic" _____mmHg
Gaaffilee amaala waliin walqabatan hirmaachuu ykn hirmaachuu dhabuu murteessan		
1	Ji'oota lamaan darban keessa seenaa dhukkuba busaa qabduu?	0.lakkii 1.eeyyee
2	Amala Sigaaraa xuuxuu qabdaa?	0.lakkii 1.eeyyee
3	Amala dhugaatii alkoolii dhuguu qabdaa?	0.lakkii 1.eeyyee
4	Gaaffii 3f eeyyee yoo ta'e guyyaatti meeqa dhugda?	0. dhugaati lama oli (hin hirmaatu/ttu) 1. dhugaati lama gadii (ni hirmaata/tti)
Maqaa nama oddeeffannoo sassabee_____Guyyaa_____Mallattoo_____		

- **Gucaa/tarreewwan sakatta'iinsaa**

Tarreewwan sakatta'iinsa kunin galmee yaalaa dhukkubsattoota dhukkuba sukkaaraa gosa 2ffaa Hospitaala Waliigalaa Bishooftuu, giddugala Itoophiyaatti argaman irraa jijjiiramoota kilinikaa walitti qabuuf qophaa'an.

Lakk	Oddeeffaannoo	Filannoowwan ta'uu danda'an
1	Yaala afaaniin fudhatamu kan sukkaara hir'isuu yeroo ammaa fayyadamaa jiru	1. 2. 3.
2	Yeroo dhukkubni sukkaaraa erga adda baafamee booda	0. waggaa torba hin guunnee, lakkoofsan ibsi____ 1. waggaa torbaa olitaheera, lakkofsan ibsi____
3	Ji'oota lamaan darban keessatti hammi sukkaara dhiiga soomanaa (mg/dl).	Ji'aa 1_____mg/dl Ji'aa 2_____mg/dl
4	Haallii fayya walxaxaa yoo xiqqatee gosti tokkoo jira?	0.lakki 1.eeyyee
Maqaa nama oddeeffannoo sassabee_____mallattoo_____guyyaa_____		

- **Gaaffilee garee waraqaa ragaa yaala fayyaatif dhufaanif fi gargaartoot waldhanmtoota kan hirmaatoota qo'annoo kana ta'anif kan qophaa'ee fi kallaatii waligala**

  Lakk adda hirmaata: _____

➢ Maaloo gaaffilee filaannoo qabaniif filaannoo keessaanin nuuf mirkaneessaa.

Lakk.	Gaaffiillee	Filaannoo
	Kutaa-I:Gaaffiwwaan haasummaa waliin walqabatan	
1.1	waggaa guutuun Umriin kee meeqa?	_____
1.2	Saala/koorniyaa	0. Dhiiraa 1.Dhalaa
1.3	Bakki jireenyaa keessan eessa?	0. Magaala 1. Baadiyyaa
1.4	Sadarkaan barnootaa keessaan maal fakkaata?	0.dubbisuu fi barreessuu hin dand'uu 1.dubbisuu fi barreessuu nan danda'a 2.sadarkaa tokkoffaa 3. sadarkaa lammaffaa 4.sadaarka olaanoo
1.5	Hojiin keessan maali?	0.Hojjii hin qabu 1.daldalaa 2.qotee bulaa 3. Hojjataa/ttu mootummaa
1.6	Haallii gaa'elaa keessanii maal fakkaata?	0. Kan hin heerumnee/fuune 1. Kan heerumtee/fuudhee 2. Kan addaan baahan 3. Kan abban warra irra darbee(du'e)ykn haati warra jalaa darbitee(duute)

1.7	Galiin ji'aa keessan (ETB)?	0. <1500 Qr.Itiyoophiyya. 1. 1500-6000 Qr.Itiyoophiyya. 2. >6000 Qr.Itiyoophiyya.
	Kutaa-II: Safartoo Anthropometric fi Dhiibbaa Dhiigaa	**Bu'aa safara qaama**
2.1	Ulfaatina Kiiloogiraamiin	_____Kg
2.2	Hojjaa qaama hirmaattota	_____m
2.3	Indeeksii ulfaatina qaamaa Kg/m2	_____Kg/m^2
2.4	Naannawa Luqqeettuu	_____cm
2.5	Naannawwa Mudhii	_____cm
2.6	Reeshiyoo naannawa mudhii fi luqqeettuu	_____
2.7	Dhiibbaa dhiigaa mmHg dhan	1."Systolic"_____mmHg 2."Diastolic" _____mmHg
Gaaffilee amaala waliin walqabatan hirmaachuu ykn hirmaach dhabuu murteessan		
1	Ji'oota lamaan darban keessa seenaa dhukkuba busaa qabduu?	0.lakkii 1.eeyyee
2	Amala Sigaaraa xuuxuu qabdaa?	0.lakkii 1.eeyyee
3	Amala dhugaatii alkoolii dhuguu qabdaa?	0.lakkii 1.eeyyee
4	Gaaffii 3f eeyyee yoo ta'e, guyyaatti meeqa dhugda?	0. dhugaati lama oli (hin hirmaatu/ttu) 1. dhugaati lama gadii (ni hirmaata/tti)
Maqaa nama oddeeffannoo sassabee_____Guyyaa_____Mallattoo_____		

አባሪ I፡ የመረጃ ሉህ

የአማርኛ ቅጂ

የጥናቱ ርዕስ፡ በቢሾፍቱ አጠቃላይ ሆስፒታል፣ የሁለተኛ ዓይነት የስኳር በሽታ ያለባቸው ጎልማሶች የፕሌትሌት ደም ዓይነት መጣን መወቅ::

ዋና ተመራማሪ ስም፡ ደረጀ አበበ

ድርጅት፡- ጅማ ዩኒቨርሲቲ (የህክምና ላቦራቶሪ ሳይንስ ት/ት ክፍል)

ስፖንሰር፡ ወልቂጤ ዩኒቨርሲቲ

መግቢያ፡ ይህ የመረጃ ፎሪም ከላይ ለተጠቀሰው ጥናት ሁሉንም መረጃዎች ይይዛል። በጥናቱ ውስጥ ስለሚከናወኑ አጠቃላይ ሂደቶች፤ አስፈላጊውን መረጃ ይሰጣል:: የእርስዎ ተሳትፎም በእርስዎ ውሳኔ ላይ የተመሠረተ ነው።

የጥናቱ መግለጫ እና ዓላማ

የስኳር በሽታ ሥር የሰደደ ኃይል ሰጪ ምግብ፣ ስብ እና ገንቢ ምግብ በበርካታ ምክንያቶች የምግብ መብላላት መዛባት ነው። ይህም በአለም አቀፍ ደረጃ እየጨመረ ሲሆን፤ በግለሰቦች፣ ቤተሰብ፤የህዝብ ጤና እና ደህንነት ላይ ትልቅ ፈተና ሆኗል።በደም ዉስጥ የስኳር ማጣን ስጫምርና ከቁጥጥር ውጭ ስሆን፤ ከጊዜ በኋላ በብዙ የሰውነት ስርዓቶች ላይ፤ በተለይም በነርቭ እና በደም ቧንቧዎች ላይ ከፍተኛ ጉዳት ያስከትላል::

የሁለተኛዉ የስኳር በሽታ ዓይነት ታማም ውስጥ የፕሌትሌት ደም ዓይነት(platelet indices) መጠን ማወቅ በመረጃ ላይ የተመሰረተ ውሳኔ ለማድረግ እና መፊተ ለመስጣጥ አስፈላጊ ነው። እኔ እስከማውቀው ድረስ በኢትዮጵያ በተለይም በዚህ የጥናት ቦታ የሁለተኛዉ ዓይነት የስኳር በሽታ ታማም(type2 diabetic patients)ላይ የፕሌትሌት ደም ዓይነት መጠን መወቅ ለይ የተደረገ ጥናት በጣም አናሳ ነው። ስለዚህ ይህ ጥናት በቢሾፍቱ አጠቃላይ ሆስፒታል፣በሁለተኛዉ ዓይነት የስኳር በሽታ ያለበቾ ጎልማሳ ታመምዎች ዉስጥ የፕሌትሌት ደም ዓይነት መጣን ለመወቅ የተዘገጀ ነዉ::

ሂደቶች

ከላይ ለተጠቀሰው ጥናት አንዳንድ ጥያቄዎች እንዳሉ ለሰዉቆት ይፈልጋሎ::ማህበራዊና አከባብያዊ ኑሮ ባህሪያት፤ሌሎች ተዛማጅ ባህሪያት እና ናሙናዎች ለላቦራቶሪ ምርመራ ይወሰዳሉ:: በጥናቱ ለመሳተፍ ፈቃደኛ ከሆኑ ለተሳትፎዎ፣ ለናሙናዎች እና ለጥናቱ የሚያስፈልጉ መረጃዎችን ለመስጠት በመረጃ ላይ የተመሰረተ ስምምነት እንዲሰጡ ይጠየቃሉ። የተሰበሰበው ናሙና በቢሾፍቱ አጠቃላይ ሆስፒታል ላብራቶሪ ክፊል እንደምመረመር ለአረጋግጥ ይወደሎ።

ስጋትና ጉዳት

የደም ናሙና በሚሰበሰብበት ጊዜ መጠነኛ ህመም እና ምቾት ማጣት ሊኖር ይችላል፤ግን ቶሎ ይጣፉል::ሁሉም ናሙናዎች የሚሰበሰቡት በመደበኛ የአሠራር ሂደቶችን (SOPS) በመከተል ነው።

ጥቅሞች እና ማካካሻዎች

ከዚህ ጥናት ምንም የገንዘብ ክፊያ እንደሌለ ማሳወቅ እፈልጋሎ። ይሁን እንጂ በላብራቶሪ ውጤቱ ላይ በመመርኮዝ ለበለጠ እንክብካቤ እና ህክምና ከህክም ጋረ እናገናኛለን::

የጥናቱ ምስጢራዊነት

የተሰበሰበውን መረጃ ሚስጥራዊነት በተመለከተ፣ መረጀሆቹ በተቆለፉ ሳጥን ውስጥ እንደሚቀመጡ እና የላበራቶር ውጤቶች ላይ የእርስዎ ስም ሳይጸፊ ልሁ ቁጥር በመጠቃም ይመሰገባል፡፡የተሰበሰበ ናሙና ለሌላ ዓላማዎች ጥቅም ላይ አይወልም፡፡

ያለ መቀበል ወይም ጥሎ የመውጣት መብት

በመጨረሻም፣ በማንኛውም ጊዜ ከጥናቱ የመውጣት ሙሉ መብት እንዳለዎት እና በእምቢታዎ ምክንያት በማንኛውም ዓይነት የጤና አገልግሎት ላይ አድልዎ እንደማይደረግዎት አሳውቃችኋለሁ።

ጥያቄ ካሉዉት

ስለ ጥናቱ ማንኛዉም ጥያቄ ወይም ቅሬታ ስኖራቸዉ የሚከተሉትን ስልኮች ወይም ኢሜል አድራሻ በመጠቀም የጥናቱን ባለቤቶች ማነጋገር እንደሚችሉ እናስወቃለን፡፡

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አባሪ II፡ የስምምነት ቅጽ

የአማርኛ ቅጽ

የተሳታፊዎ ስም _____

ከላይ ስለተጠቀሰው የምርምር ዓላማ በምረዳው ቋንቋ ሙሉ መረጃ አግኝቻለሁ። በቢሾፍቱ አጠቃላይ ሆስፒታል፣የሁለተኛዉ ዓይነት የስኳር በሽታ ያለባቸው ጎልማሶች ዉስጥ የፐሌትሌት ደም ዓይነት መጥን መዉቅ በምሎ ለጥናት ናሙና እንደሚወሰድ እና ቃል መጠይቅ እንደሚደረግ ተነግሮኛል። በተጨማሪም፣ በምርምር ይደት ውስጥ የሚሰበሰቡት መረጃዎች በሙሉ በሚስጥር እንደሚጠበቁ ተነግሮኛል። በጥናቱ ለመሳተፍ ወይም ለመካፈል ፈቃደኛ ካልሆንኩ የአሁኑ እና የወደፊት የሐኪምና አገልግሎቴ እንደማይጎዳ ተረድቻለሁ።

እስማማለሁ_____አልስማማም_____

ስለዚህ በዚህ ጥናት ውስጥ ለመሳተፍ በነፃነት ፈቃዴን እሰጣለሁ።

የተሳታፊው ስም፡_____ፊርማ_____ ቀን_____

የተመራማሪው ስም፡ _____ ፊርማ _____ ቀን_____

አባሪ III፡ መጠይቆች

የአማርኛ ቅጂ

- **<u>ለሁለተኛዉ ዓይናት የስኩር በሽታ ታማምዎች የተዘገጀ ጥያቀዎች</u>**

የተሳታፊዉ ካርድ ቁጥር _____

የተሳታፊዉ ኮድ _____

<u>አጠቃላይ መመሪያ :</u>ለሁሉም ጥያቄዎች አማራጮች ያሉት ሲሆን እርሶ ተገቢ ነዉ በሚሉት ላይ ያክብቡ፡፡

ተ.ቁ	ጥያቄዎች	ሊሆኑ የሚችሉ መልሶች
	ክፍል I:: ማህበራዊና አከባብያዊ ኑሮ ባህሪያት	
1.1	ዕድሜ በዓመት	_____
1.2	ጾታ	0. ወንድ 1. ሴት
1.3	ያሚኖሩባት ቦታ	0. ከተማ 1. ገጠር
1.4	የትምህርት ደረጃዎ?	0. ማንበብ እና መጻፍ አልችልም 1. ማንበብ እና መጻፍ ይችላሎ 2.የመጀመሪያ ደረጃ ት/ት ቤት 3. ሁለተኛ ደረጃ ት/ት ቤት 4.ከፍተኛ ትምህርት ደረጃ
1.5	የስራ ማስክ	0. ሥራ አጥ 1. ነጋዴ 2. ገበሬ 3. የመንግስት ሰራተኛ
1.6	የትዳር ሁኔታ	0. አላገባሁም 1. ባለ ትዳር ነኝ 2. ፈትቼሃለሁ 3. ባሌ ሞቶዋል/ምስቴ ሞታለች
1.7	ወርሃዊ ገቢዎ በኢትዮጵያ ብር?	0. <1500በር 1. 1500-6000በር 2. >6000ብር
	ክፍልII. አንትሮፖሜትሪክ እና ደም ግፊት መለኪያዎች	

2.1	ክብደት	_____ኪ.ግ
2.2	ቁመት	_____ ሜ
2.3	የሰውነት ክብደት ኢንዴክስ	_____ ኪ.ግ/ሜ^2
2.4	የመቀመጫ ዙሪያ በሴንቲሜትር	_____ሴ.ሜ
2.5	የወገብ ዙሪያ በሴንቲሜትር	_____ሴ.ሜ
2.6	ወገብ ዙሪያ ለ መቀመጫ ዙሪያ	_____
2.7	የተሳታፊዎች የደም ግፊት በ ሚሊሜትር ሜርኩሪ	1. ሲስቶሊክ_____ሚሊሜትር ሜርኩሪ 2.ዲያስቶሊክ_____ ሚሊሜትር ሜርኩሪ
	ክፍል III. ክሊኒካዊ እና ባህሪ ባህሪያት (ተሳታፊዎችን ለማግለል ወይም ለማካተት)	
1	ባለፉት ሁለት ወራት የወባ በሽታ ታሪክ አለህ?	0.አይ 1.አዎ
2	ሲጋራ ማጨስ ልማድ አለህ?	0.አይ 1.አዎ
3	አልኮል የመጠጣት ልማድ አለህ?	0.አይ 1.አዎ
4	ለጥያቄ3 አዎ ከሆነ፣ በቀን ምን ያህል ይጠጣሉ?	0. ከሁለት መጠጥ በላይ (በምርምር ውስጥ አይሳተፍም) 1. ከሁለት መጠጥ በታች (በጥናት ውስጥ ይካተታሉ)
የመረጃ ሰብሳቢው ስም _____ ቀን_____ፊርማ_____		

❖ **የፍተሻ ዝርዝሮች(ቼክ ሊስት)**

እነዚህ ቼክ ሊስት በቢሾፍቱ አጠቃላይ ሆስፒታል ከሚከታተሉት የሁለተኛዉ ዓይነት የስኳር ታመምዎች የህክምና መረጃዎቹ ለጥናቱ የምአስፈልጉ ለመሰብሰብ የተዘጋጀ ነዉ።

ተ.ቁ	የህክምና መራጀዎች	ሊሆኑ የሚችሉ አማራጮች
1.1	ለሁለተኛዉ አይነት የሱኳር በሽታ የምስጥና የምዋጥ መደንት አይነት	1. 2. 3.
1.2	በስኳር በሽታ ከተያዘ ጀምሮ ስንት አማት ነዉ	0. <7 ዓ. ም ፤በዓማት _____ 1. > 7 ዓ. ም፤በዓመት_____
1.3	ባለፉት ሁለት ወራት ውስጥ የሱኳር መጣን	ወር 1፡_____ mg/dl ወር 2፡_____ mg/dl
1.4	የተመምዉ የጠና ዉስብስብነት ብያንስ አንድ ከአለ?	0.አይ 1.አዎ
የመረጃ ሰብሳቢው ስም _____ ፊርማ _____ ቀን_____		

- **ለህክምና ምርመራ ለመጡ እና የታካሚ ረዳት ተሳታፊዎች**

የተሳታፊዉ ኮድ፡_____

አጠቃላይ መመሪያ

- ለሁሉም ጥያቄዎች አማራጮች ያሉት ሲሆን እርሶ ተገቢ ነዉ በሚሉት ላይ ያክብቡ ፡፡

ተ.ቁ	ጥያቄዎች	ሊሆኑ የሚችሉ መልሶች
	ክፍል I: ማህበራዊና አከባብያዊ ኑሮ ባህሪያት	
1.1	ዕድሜ በዓመት	_____
1.2	ጾታ	0. ወንድ 1. ሴት
1.3	ያሚኖሩባት ቦታ	0. ከተማ 1. ገጠር
1.4	የትምህርት ደረጃዎ?	0. ማንበብ እና መጻፍ አልችልም 1. ማንበብ እና መጻፍ ይችላሎ 2.የመጀመሪያ ደረጃ ት/ት ቤት 3. ሁለተኛ ደረጃ ት/ት ቤት 4.ከፍተኛ ትምህርት ደረጃ
1.5	ያስራ ማስክ	0. ሥራ አጥ 1. ነጋዴ 2. ገበሬ 3. የመንግስት ሰራተኛ
1.6	የትዳር ሁኔታ	4. አላገበሁም 5. ባለ ትዳር ነኝ 6. ፈትቼሃለሁ 7. ባሌ ሞቶዋል/ምስቴ ሞታለች
1.7	ወርሃዊ ገቢዎ በኢትዮጵያ ብር?	0. <1500ብር 1. 1500-6000ብር 2. >6000ብር
	ክፍል II. አንትሮፖሜትሪክ እና ደም ግፊት መለኪያዎች	
2.1	ክብደት	_____ኪ.ግ
2.2	ቁመት	_____ ሜ

2.3	የሰውነት ክብደት ኢንዴክስ	_____ ኪግ/ሜ^2
2.4	የመቀመጫ ዙሪያ ክበብ በሴንቲሜትር	_____ሴ.ሜ
2.5	የወገብ ዙሪያ ክበብ በሴንቲሜትር	_____ሴ.ሜ
2.6	የወገብ ዙሪያ ለ መቀመጫ ዙሪያ	_____
2.7	የተሳታፊዎች የደም ግፊት በሚሊሜትር ሜርኩሪ	1. “ሲስቶሊክ _____ሚሊሜትር ሜርኩሪ 2. “ዲያስቶሊክ _____ በ ሚሊሜትር ሜርኩሪ
	ክፍል II. ክሊኒካዊ እና ባህሪ ባህሪያት (ተሳታፊዎችን ማግለል ወይም ማካተት)	
1	ባለፉት ሁለት ወራት የወባ በሽታ ታሪክ አለህ?	0.አይ 1.አዎ
2	ሲጋራ ማጨስ ልማድ አለህ?	0.አይ 1.አዎ
3	አልኮል የመጠጣት ልማድ አለህ?	0.አይ 1.አዎ
4	ለጥ3 አዎ ከሆነ፣ በቀን ምን ያህል ይጠጣሉ?	0.ከሁለት መጠጥ በላይ (በምርምር ውስጥ አይሳተፍም) 1.ከሁለት መጠጥ በታች (በምርምር ውስጥ ይሳተፍል)
የመረጃ ሰብሳቢው ስም _____ ቀን_____ፈርማ_____		

Annex IV: Laboratory procedure

- **Specimen collection**

- **Venous blood collection**

a) Needed materials

- Glove
- 70% alcohol
- Tourniquet
- EDTA test tube
- Serum separator test tube
- Sterile Syringes
- Gauze pads or cotton
- Marker
- Rack
- Sharp container
- Band-aid

b) **Procedure**

1. All necessary materials and equipment were arranged
2. The right patient was identified and allowed to sit comfortably in an armchair stretching his/her arm.
3. "The tourniquet was applied as needed"
4. The vein was selected for puncture
5. "The site was cleaned with a gauze pad or cotton moistened with 70% alcohol".
6. The needle was taken and its cover was removed and inspected
7. anchor vein was checked with the thumb
8. The needle was inserted into the vein with the bevel faced up and the sample was collected and the tourniquet was released
9. The needle was withdrawn from the vein and disposed
10. Blood samples dispense on the wall of K2-EDTA test tubes & mixed well, dispense in an organ tube leave it for 15 minutes, and do not mix blood samples with tube gel.
11. Finally, test tubes were labeled with an ID number or unique code.

❖ Thin blood film preparation

i. Needed materials

- Clean microscope slides
- Well-mixed EDTA blood sample
- Pipette
- pencil
- Gloves
- Waste and sharps disposal containers

ii. Procedures

- A drop of EDTA blood was placed approximately 1.0 cm far from the end of the glass slide.
- The spreader slide was placed in front of the drop of blood at an angle of about 30^{o}-40 ^{o} to the slide and then was moved back to make contact with the drop
- The drop was spread out along the line of contact of the spreader with the slide
- The spreader was advanced with a smooth steady motion so that a thin film of blood is spread over the slide
- Then slides were labeled with the patients' ID
- Finally allowed smeared slide to air-dry

❖ **Wright staining and examination**

Procedure

- The air-dried smear film has placed the side up on a staining rack
- The smear was covered with an undiluted filtered stain and left for 3 minutes
- An equal volume of distilled water was added (i.e., the same number of drops as the stain)
- Mix by blowing until a metallic sheen appears.
- Allowed the diluted stain to act for 7 minutes
- The stain was washed off with running tap water
- The back of the slide was cleaned and stood in a rack for the smear to dry
- Oil immersion objective used for studying the fine details of the cell morphology.
- A morphology study was performed for the rechecked purpose of the CBC machine printout of platelet count, its arrangement, and for participants' malaria-caused parasite screening.

Interpretation: The slides were well-examined for malaria cause parasites. For platelet morphology, platelet size was interpreted under the microscope by the assumption of comparing platelet diameter to red blood cell diameter. We investigated three important features of platelet morphology. This includes estimating platelet numbers per field, size, and arrangement (Clumping, Satellitism).

Larger platelets: look under a microscope when platelets are approximately the same as the diameter of red blood cells. **Normal platelets:** under the microscope, when the average platelet size is normal. Platelets are approximately 2-3 microns in diameter.

Giant platelets: when under a microscope the size of platelets is approximately larger than the diameter of the red blood cells or small lymphocytes nucleus.

Quality management: Wright's stain was filtered through filter paper each day. The quality of wright's staining was checked by staining slides prepared from samples with known normal platelet counts and other slides prepared from known thrombocytopenia.Accuracy was assured, slides were cross-checked by a senior medical laboratory technologist and some slides were cross-checked by a pathologist blinded to the previous results.

- **Complete blood count by using Sysmex-xn550**

Principle: The Sysmex-XN-550 is an automated in vitro diagnostic hematology analyzer for determining whole blood diagnostic parameters. This device performs hematological analysis based on a combination of hydro-dynamically focused impedance measurements, photometry, and flow cytometry.

Hydro-dynamically Focused Impedance method: Utilizing this principle, the analyzer quantifies and measures the volume distribution of cells by measuring the changes in electrical impedance when particles suspended in conductive liquids pass through trifling holes. As each particle passes through the orifice, the electrical impedance of blood cells in the liquid changes. The changes in electrical impedance are converted into electrical impulses. The number of pulses in the analyzer is then equal to the number of passages of particles in the aperture. On the other hand, the pulse intensity is proportional to the particle volume. The electron distribution enables the separation of the RBC and PLT, lytic reactions lyses RBC, and selectively measure WBC.

Flow cytometer methods: Used for measurement of leukocyte five-differential count. A focused laser is used to illuminate a stream of WBC suspended in an optically clear diluent moving across the flow cell. A detector measures the intensity of the scattered laser light as it flows through the path of a laser beam. This is proportional to the cell volume and the complexity of the internal of the cell. The five-part total population identification is based on the analysis of the two-dimensional volumes and internal complexity distributions. Cells with a larger size or more granules tend to produce a high amount of light scattering. The external complexity and scattering angle magnitude are low, and the internal complexity increases the diffraction angle. The optical signal is used to quantify the intensity of scattered light.

Photometric principle: The Sysmex-550 analyzer uses this principle to measure hemoglobin. This reagent lyses RBC resulting in the coming out of hemoglobin. Hemoglobin concentration is measured at a specific wavelength of the analyzer in the WBC chamber. To minimize the effects of the liquid refraction and incident light, hemoglobin concentration can be determined from the difference between blank and sample measurements. At the same time, the hematocrit (HCT) is measured as a ratio of the total red blood cell volume to whole blood using the RBC pulse height detection method.

Specimen

- **Required specimen**

- Whole blood anti-coagulated with a potassium EDTA is preferred.

- Sodium Citrate may be used when EDTA platelet clumping or platelet satellitium is noted on the EDTA specimen. Platelet counts, immature platelet fraction and WBC counts are the only parameters that may be resulted from the Sodium Citrate specimen.

- **Specimen volumes required**

- Optimal draw is a tube drawn to capacity. The collection tube must be filled to a minimum of one-half full for acceptable results.

- A minimum of 1 mL of whole blood is required for sample analysis.

- An EDTA raised bottom microtainer filled above the 250 uL line is adequate. To maintain the proper anticoagulant ratio it is preferred to fill to the 250 uL line at the time of collection.

- **Unacceptable specimens including those listed below must be redrawn:**

- Clotted samples or those containing clots or fibrin strands. All microtainer specimens will be checked for clots prior to sampling by the analyzer.

- Samples drawn above an IV set

- Characteristics that may affect test results

    - Lipemia (may falsely increase HGB)

    - Icterus (may falsely increase HGB)

    - Cold agglutinins (may falsely increase WBC count, MCV and MCHC; may falsely decrease HCT & RBC count)

    - Severe hyponatremia (decreased plasma sodium level) may falsely decrease HCT causing a falsely increased MCHC.

- **Stored Specimen Stability**

- If stored at 4-8 ^{0}C within 6 hours of collection, EDTA blood samples with normal results may be analyzed up to 48 hours without significant loss of differential stability.

- Slides for a manual differential must be assessed for cellular integrity prior to reporting. If cellular integrity is not intact a manual differential or morphology should not be reported.

- Sample stability at room temperature is 8 hours. Samples stored at room temperature may exhibit an increase in MCV after 24 hours; this may be minimized by refrigeration.

- Allow refrigerated samples to come to room temperature for 30 minutes and mix by hand inversion before analysis.

Reagents for sysmex-xn550

- **DILUENTS (CELLPACK DCL):** Whole blood diluent used in hematology analyzers.

- **SULFOLYSER (SLS):** Reagent for the automated determination of hemoglobin concentration of blood. Sulfolyser is a lysing reagent that releases the hemoglobin to be measured by the SLS hemoglobin method.

- **LYSERCELL WDF:** Reagent product to be combined and used with Fluorocell WDF. By hemolyzing red blood cells with Lyser cell WDF and dying, the white blood cell component with Fluorcell WDF the counts and percentages of neutrophils, lymphocytes, monocytes, eosinophil and basophils are analyzed.
- **CELLCLEAN AUTO:** Detergent for fully automated hematology analyzers. To be used as a strong alkaline detergent to remove lysing reagents, cellular residuals, and blood proteins remaining in the hydraulics of the analyzer on XN Series/XN-L Series automated hematology analyzers.

QUALITY CONTROL:

Quality control is performed in order to monitor an analyzer's performance over time. XN-L CHECK is the material divided into (low, medium and high) used to monitor the performance of the XN-550 analyzer. Quality control should be run in accordance with regulatory agency requirements.

XN-550 Acceptable Background Counts	
Parameters	**Acceptable Limit**
WBC	0.10 x 10^3 / uL
RBC	0.01 x 10^6 / uL
HGB	0.10 g/dL
PLT	0.10 x 10^3 / uL

- **Fasting Serum glucose determination by using Cobasc311**

Principle: Glucose is measured from serum using the principle of photometric analytical measurement. Glucose is the major carbohydrate present in the peripheral blood. The enzyme hexokinase (HK) catalyzes the reaction between glucose and adenosine triphosphate (ATP) to form glucose-6-phosphate (G-6-P) and adenosine diphosphate (ADP).

In the presence of nicotinamide adenine dinucleotide (NAD), G-6-P is oxidized by the enzyme glucose-6-phosphate dehydrogenase (G-6-PD) to 6-phosphogluconate and reduced nicotinamide adenine dinucleotide (NADH). The increase in NADH concentration is directly proportional to the glucose concentration and can be measured spectrophotometrically at 340nm.

Specimen: Serum specimens must be separated immediately after clotting.

Serum: Glucose stability in samples is affected by storage temperature, bacterial contamination, and glycolysis. Plasma or serum samples should be separated from the cells or clots within 30 minutes after collection. Specimens that cannot be separated from the cells within 30 minutes should be placed on ice or refrigerated.

Reagent/Materials:

R1 MES buffer: 5.0 mmol/L, pH 6.0; Mg2+: 24 mmol/L; ATP: >4.5 mmol/L; NADP: >7.0 mmol/L; preservative.

R2 HEPES buffer: 200 mmol/L, pH 8.0: Mg2+: 4 mmol/L; HK (yeast): >300 ukat/L; G-6-PDH (E.coli): >300 ukat/L; preservative

Calibrators	
Deionized water is used automatically by the instrument as the zero calibrator.	
Calibration mode	Linear regression

Quality control and frequency:

Two levels of Quality Control should be performed at a minimum:

1. Once every twenty-four hours
2. If a new cassette of reagent is put in use
3. If a calibration is performed

❖ **Card method: C-reactive protein→for control group**

Definition: A C-reactive protein test is a blood test used to measure the amount of CRP in the blood. This test was used to screen the inflammatory status of control groups for these were our study participants. Based on this Ab test, participants were screened and included in the study.

Principle: The C-reactive protein test is based on the principle of the latex agglutination.

Procedure for card CRP (qualitative test)

- The serum was separated whole blood by Sorvall-ST-16 centrifuge which is working with 3600rpm per 3 minutes.
- All reagents and serum sample was arranged with room temperature
- Internal quality control for reagent was done using positive and negative controls
- Then One drop of serum was taken on the reaction circle of card
- then add one drop of C-reactive protein reagent was added on serum containing circle of card
- Mix with sticks and spread the fluid over the entire area of the card
- tilt the card back and forth slowly for one to two minutes and look for agglutination and interpreted

Interpretation

- Reactive/positive→agglutination of latex particles indicating the presence of CRP at significant and detectable level.
- Nonreactive/Negative→no agglutination

Annex V- Laboratory Result Reporting Format
Form for hematological parameter of Bishoftu Hospital

ID _____Age_____ Sex_____ Physician Name _____Sign____

Test	Results	Reference Range	Histogram
RBC	10^6/uL		The histogram is for RBC,WBC,PLT
HGB	gm/dl		
HCT	%		
MCV	Fl		
MCHC	gm/dl		
MCH	Pg		
RDW-SD	Fl		
RDW-CV	%		
PLT	10^3/µl		
PDW	fl		
MPV	fl		
P-LCR	%		
PCT	%		
WBC	10^3/µl or%		
NET	10^3/µl or%		
LYMPH	10^3/µl or%		
MONO	10^3/µl or%		
EON	10^3/uL or%		
BASO	10^3/uL or%		

Reporting form for peripheral morphology examination

ID_____ Age_____Sex_____Date _____

Physician Name _____

Platelet series_____

RBC series _____

WBC series _____

Possible conclusion _____

Annex VI: Declaration Sheet
Declaration

The undersigned declares that this thesis complies with the university's regulations and meets the accepted standards concerning originality and quality. Principal investigator also agrees to take responsibility for the research project's scientific, ethical, and technical conduct and the provision of required progress reports.

MSc candidate student name: Dereje Abebe

Signature: _____Date of submission: _____/_____/_____

APPROVAL OF THE ADVISORS

This research paper approved by University advisors:

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Examiner

Name: _____Signature: _____Date_____/_____/_____

Name: _____ Signature: _____Date_____/_____/_____

Name of School head: _____

Signature: _____ Date of submission: _____/_____/_____